VOLUME 72 *September, 1955* NUMBER 3

THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

OFFICIAL JOURNAL OF AMERICAN TRUDEAU SOCIETY
MEDICAL SECTION OF NATIONAL TUBERCULOSIS ASSOCIATION

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Published Monthly at Mount Royal and Guilford Avenues, Baltimore 2, Maryland, by the National Tuberculosis Association • Business Off., 1790 Broadway, New York 19, N. Y.

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THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES

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Subscription: The subscription price of the REVIEW is $12 for the calendar year. Checks should be made payable to Collier Platt, Treasurer. The price of single copies is $1.50 postpaid.

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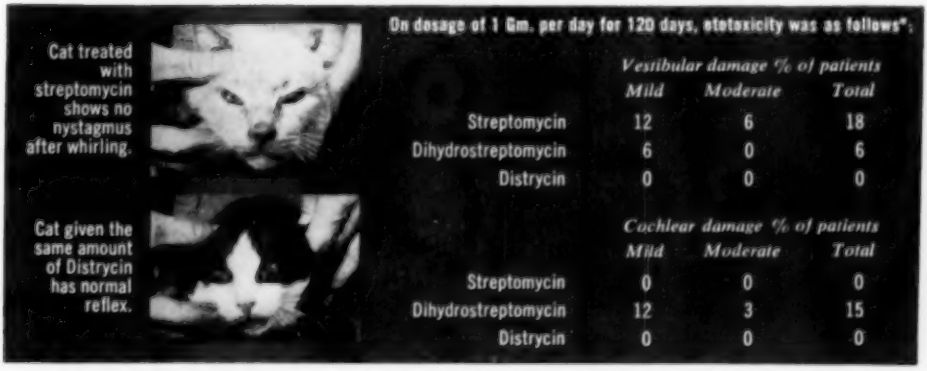
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*Heck, W.E.; Lynch, W.J., and Graves, H.L.: Acta oto-laryng. 41:416, 1953.

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VOLUME 72 *September, 1955* NUMBER 3

THE MANAGEMENT OF SPONTANEOUS PNEUMOTHORAX[1, 2]

ALBERT G. MARRANGONI, CLIFFORD F. STOREY, AND PHILIP O. GEIB

(Received for publication February 15, 1955)

Introduction

Spontaneous pneumothorax is a relatively common disorder which has been recognized by physicians for a great many years. It was formerly thought to be due exclusively to tuberculosis. The studies of Kjaergaard (1) in 1932, however, and of numerous subsequent writers who have reported large series of cases of spontaneous pneumothorax (2–5) have done much to dispel the idea of tuberculosis as a common cause of this condition. Healthy subjects are usually affected, and expectant treatment may result in long periods of disability and hospitalization, with an occasional "captive lung," pleural effusion, or empyema. Simultaneous bilateral spontaneous pneumothorax is not exceptionally rare, and deaths have been reported in this situation as well as from severe untreated unilateral tension pneumothorax.

The treatment of spontaneous pneumothorax has been greatly altered in the past decade, but there is still some confusion and disagreement concerning the proper management of patients with this condition. The rather prolonged period of inactivity which conservative treatment usually entails and the occasional major complication associated with it have prompted the consideration of other forms of therapy. The purpose of this report is to analyze a series of 80 patients with spontaneous pneumothorax who were managed by one of several methods and to describe the therapeutic program which the present writers have found to be the most satisfactory and successful.

Observations

General background of group

The patients in the present series were admitted to the U. S. Naval Hospital, Portsmouth, Virginia, between January 1, 1952, and April 1, 1954. The material consisted of 80 cases collected from the hospital records, of which 25 unselected

[1] From the U. S. Naval Hospital, Portsmouth, Virginia.

[2] The opinions or assertions expressed in this paper are private ones of the authors and are not to be construed as official or to reflect the views of the Navy Department or the naval service at large.

TABLE 1

OCCURRENCE OF SPONTANEOUS PNEUMOTHORAX ACCORDING TO AGE

Age	Number of Cases	Percentage
17–20	17	21.25
21–30	49	61.25
31–40	13	16.25
41–50	1	1.25
Total	80	100.00

patients were treated on the thoracic surgery service. All of these 80 patients were otherwise healthy persons who developed spontaneous pneumothorax in the absence of neoplastic disease, active tuberculosis, or trauma. There were 78 men and 2 women in the series. This sex distribution has no significance, since the vast majority of patients admitted to this military hospital are men. The distribution according to age is presented in table 1.

The majority of patients were between twenty and thirty years of age (61.25 per cent), the extremes being nineteen and forty-eight years. Although there is some age selectivity in patients admitted to military hospitals, the above figures closely parallel the generally reported age incidence of spontaneous pneumothorax. In the 80 cases reported herein, both sides of the chest were involved with equal frequency. This agrees with the experience of Hyde and Hyde (2), but other writers (6, 7) have reported a slightly higher incidence on the right side.

CLINICAL AND ROENTGENOGRAPHIC MANIFESTATIONS

Symptoms were present in all cases. Sharp pleuritic chest pain on the side of collapse was the presenting symptom of 77 patients. It was usually localized to the anterior chest wall. Associated shoulder pain was not uncommon. In 2 patients there was numbness in the arm of the involved side. Dyspnea was present in 72 cases, nonproductive cough in 12, and both pain and dyspnea were the complaints of 69 patients. One patient with bilateral pneumothorax was cyanotic on admission. Fever occurred in 7 patients. Two other patients had pneumonia on the side of their collapse.

In general, the symptoms were pronounced when the degree of collapse was 60 per cent or more. Conversely, with lesser degrees of collapse, the symptoms were usually minimal.

In almost all instances the patients were carrying out their normal activity at the time of onset of their symptoms. Vigorous physical exertion did not appear to predispose to or precipitate spontaneous pneumothorax. The symptoms were generally of short duration, but 2 patients had had symptoms for several days before admission.

Although numerous complications or sequelae have been reported by others (2, 3, 6, 7), no instance of "captive lung" or empyema occurred in this series of cases. This may be attributed, in part at least, to the fact that these service personnel were hospitalized and under medical surveillance from the time of diagnosis until complete re-expansion occurred.

The extent of collapse at the time of admission was estimated in each case on the basis of chest roentgenograms. Collpase of the affected lung was less than 20 per cent in 8 cases, between 20 and 50 per cent in 27 cases, and 50 per cent or greater in 45 cases. There was no correlation between the extent of collapse and the duration of symptoms prior to admission.

Recurrent collapse occurred in 12 patients (15 per cent of the entire group of 80 cases). Other writers (2, 3, 5, 7) have reported an incidence of 9 to 27 per cent of recurrent pneumothorax. All recurrences in the present series occurred in patients who were treated conservatively. There has been no case of recurrent collapse among the 25 patients treated by thoracoscopy, intrapleural talc poudrage, and prompt pulmonary re-expansion by active suction.

Simultaneous bilateral pneumothorax was observed in one patient. She was treated by the aggressive method to be described. This woman also had a bloody effusion on one side at the time of her admission. A serous or serosanguineous pleural effusion of significant amount was noted in 8 of the 55 patients treated by the more conservative methods. Among the patients treated by thoracoscopy and poudrage, it is not uncommon for a small amount of serous fluid to be withdrawn through the intercostal catheter. This undoubtedly represents a response to the irritating properties of the talc which has been introduced. Since it is withdrawn as rapidly as it forms, these patients do not develop an intrapleural fluid collection and in no case has it been necessary to do a thoracentesis following the withdrawal of the catheter, which is ordinarily done forty-eight hours after its insertion. At the time of their discharge, patients treated by the active method almost invariably exhibit sharp costophrenic angles, and the pleural reaction is usually not sufficient to be visible on the chest roentgenograms.

There was no record of a clinically significant tension pneumothorax in any of the 80 patients in this series, although numerous patients showed total collapse of the lung on the involved side. This finding may be a reflection of the promptness with which these patients were hospitalized and placed on strict bed rest or more active therapy.

ETIOLOGY

Spontaneous pneumothorax is obviously due to entry of the air into the pleural space. However, the manner in which the disruption of the integrity of the visceral pleura occurs has been a subject of much controversy. The etiologic factors include diseased pleura or adjacent lung, acute bacterial infections, neoplastic invasion, and pulmonary or mediastinal emphysema. Functional obstruction due to such conditions as bronchospasm, mucosal edema, and infection has also been followed by peripheral alveolar inflation, bleb formation, and rupture.

The stigma of tuberculosis has previously been attached to patients with this disorder because many of the earlier reports of spontaneous pneumothorax were from tuberculosis services. It is well known that spontaneous pneumothorax is not a rare complication of far advanced tuberculosis (8, 9). On the other hand, tuberculosis is, percentagewise, a rather *uncommon* cause of spontaneous pneumothorax. The chest roentgenograms of each patient in the present series were carefully studied for evidence of the presence of active or healed tuberculosis, but no

patient presented roentgenographic evidence of that disease. In addition, at the time of thoracoscopy the visceral pleural surfaces were carefully inspected for subpleural tubercles or other findings suggestive of tuberculosis. When such pathology was noted, talc poudrage was not carried out.

Role of blebs: The role of rupture of subpleural blebs or bullae in the production of pulmonary collapse was first described by Hayashi (10) and subsequently confirmed by many others (2, 4, 6). Brock (11) found fifty instances of generalized or localized bullous emphysema in a series of seventy-one patients examined by chest roentgenograms and/or thoracoscopy. In the present series, 23 patients were examined by thoracoscopy and bullous emphysema was noted in 13 of them. In addition, pleural tears at the base of old adhesions were visualized in 4 other patients. Blebs or bullae were visualized in the chest roentgenograms of 5 of the 55 patients who were not examined by thoracoscopy. In the present writers' experience, chest roentgenograms are notoriously unreliable in the diagnosis of small subpleural emphysematous blebs.

It is now the consensus that spontaneous pneumothorax usually results from the rupture of some cystlike lesion of the lung. The visceral subpleural space is occupied by a layer of lung tissue that consists of potential spaces which have the ability to increase into cystlike dilatations, since they are in direct communication with the alveoli or bronchioles. These constitute a potential source of tension or valvular pneumothorax should they distend and rupture. Hamman (12) has pointed out that spontaneous collapse of the lung may also result from the rupture of the mediastinal pleura in spontaneous mediastinal emphysema.

TREATMENT

Patients presenting severe symptoms of tension pneumothorax, bilateral pneumothorax, a large hydro- or hemopneumothorax, or pneumothorax superimposed on an already seriously impaired pulmonary function are obviously emergency problems and everyone agrees that they require immediate active therapy. In other patients, the matter of loss of time to the patient as well as the possibility of recurrence should the lung re-expand without active therapy, or "captive lung" if it does not do so, would seem to justify an aggressive form of treatment. Therefore, it has become the practice of the present writers to treat all cases of spontaneous pneumothorax as surgical emergencies.

Cannula Treatment

It is the current practice in this hospital to take the patient with spontaneous pneumothorax to the operating room immediately after admission and to introduce a trochar and cannula into the pleural space under local infiltration anesthesia. The site of insertion varies in individual cases, but as a rule the second or third interspace in the anterior axillary line or the fourth or fifth interspace in the midaxillary line is used. The trochar is withdrawn and a thoracoscope is introduced through the cannula so that the surface of the collapsed lung can be examined. After careful inspection has been carried out and the underlying pathology determined, the thoracoscope is withdrawn and the visceral and

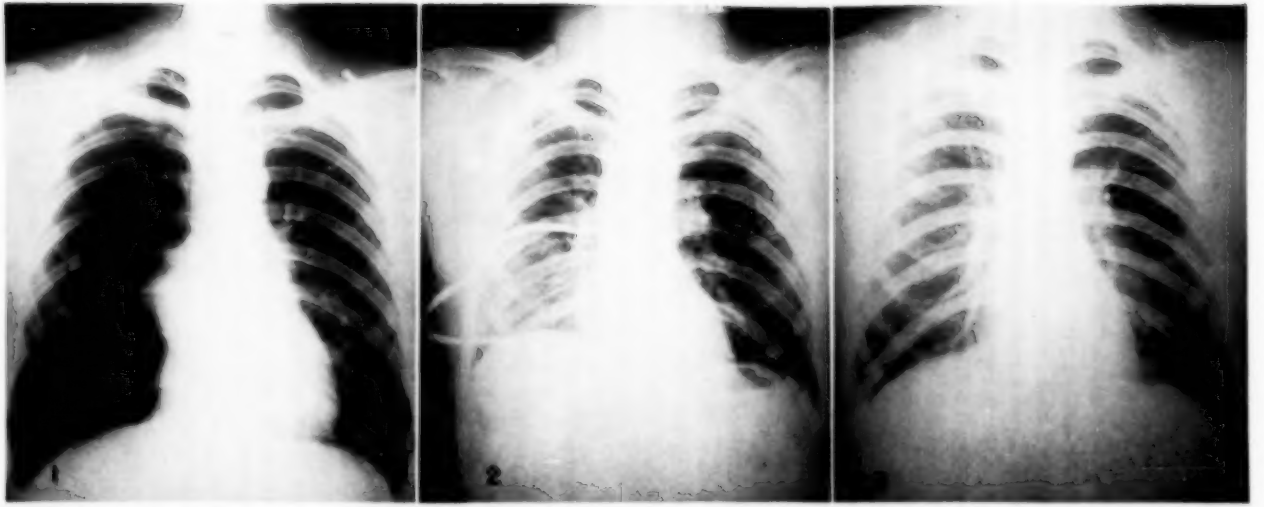

FIG. 1. Postero-anterior chest roentgenogram showing complete collapse of right lung at time of admission.

FIG. 2. Postero-anterior chest roentgenogram showing complete re-expansion of the right lung immediately following thoracoscopy, talc poudrage, and active underwater seal catheter suction.

FIG. 3. Postero-anterior chest roentgenogram showing complete re-expansion of right lung at time of discharge, three days after admission.

parietal pleurae are thoroughly poudraged with sterile talc which is insufflated by means of a powder blower. The long stem of the insufflator is inserted through the cannula and pointed in various directions as the powder is blown into the pleural space so as to thoroughly cover the pleural surfaces. A No. 16 French red rubber catheter is then placed through the cannula into the pleural space, the cannula is withdrawn, and active underwater seal suction at a negative pressure of 15 to 20 cm. of water is begun.

These patients are returned from the operating room to their rooms by way of the radiology department, where a postero-anterior chest film is obtained. This almost always shows complete re-expansion of the lung (figures 1 and 2).

Active suction is maintained for twenty-four hours, and the patient is then placed on simple underwater seal drainage for an additional twenty-four hours. If the lung remains fully expanded and there is no fluid collection, the catheter is removed (figure 3). Penicillin is given intramuscularly as long as the intercostal catheter is in place.

Twenty-five of the 80 patients in the present series were treated in this active manner.

Conservative Methods

Fifty-five of the 80 patients were treated conservatively by one of three methods. The most common treatment consisted of simple bed rest, and this measure was used in the large majority of these 55 patients. Much less commonly, repeated needle aspiration was used in addition to bed rest. In 2 patients, a catheter was inserted and simple underwater seal without active suction was utilized. The time required for full re-expansion, using various methods of treatment, is summarized in table 2.

Results

The average time necessary for complete re-expansion in the 25 patients treated by the active method described and advocated was a matter of minutes, as compared with the more conservative measures, which required an average of from 22.5 days to 47 days, depending upon the method used. The over-all average time required for re-expansion in the 55 conservatively treated patients was 30 days. The average period of hospitalization of these patients managed conserva-

TABLE 2

Results Obtained in 80 Cases of Spontaneous Pneumothorax by Various Methods of Treatment

Method	Number of Patients Treated	Days for Expansion		
		Longest	Shortest	Average
Bed rest	49	70	8	29
Needle aspiration	4	60	37	47
Aspiration plus water seal	2	23	22	22.5
Catheter plus active underwater-seal suction	25	Immediate	Immediate	Immediate

tively was 43 days. The average period of hospitalization required by those patients managed by thoracoscopy, poudrage, and immediate active suction was three days.

Discussion

The rapid recovery achieved by the program of active treatment of spontaneous pneumothorax is of great importance to the patient, for it represents an economic saving which could not be achieved by other forms of therapy. The point is well illustrated by the experience with the 80 cases reported herein. The 55 patients who were treated by conservative methods required an average period of hospitalization of 43 days per patient, or a total of 2,365 man-days on hospitalization for the patients in this group. On the other hand, the 25 patients managed by immediate thoracoscopy examination, talc poudrage, and active intercostal suction required an average of but three days' hospitalization per patient, or a total of 75 hospital days for this series of actively treated patients. If it may be assumed, as seems reasonable, that the 55 unselected conservatively treated patients would have responded equally as promptly to the energetic method of treatment described herein as did the 25 patients who were so treated, a saving of 2,200 man-days of hospitalization could have been affected. The result would have been a great saving to the individual patient and would have permitted much more efficient utilization of the available hospital beds. In view of the present high cost of hospitalization and the current shortage of hospital beds, these factors assume considerable significance. It is also noteworthy that some of the 55 conservatively treated patients included in this report represented cases of recurrent pneumothorax, so that actually the length of hospitalization per individual patient was even greater than that which has been indicated. To be sure, it is realized that in civilian practice it is not necessary, as a rule, to retain patients with spontaneous pneumothorax in the hospital until complete pulmonary re-expansion has been achieved. Nevertheless, such patients normally are not allowed to return to work until this has occurred. Furthermore, the occasional major surgical operation required in the treatment of an empyema or an imprisoned, unexpandable lung increases greatly the expense and delays the patient's return to gainful employment. Finally, a significant percentage of recurrences can be anticipated in the conservatively treated patients. As previously mentioned, there has been no recurrence among the 25 reported patients treated actively in the manner described.

Thoracoscopy is a relatively benign operative procedure which not only aids in determining the underlying pathology, but may provide other interesting and helpful information as well. In some cases, roentgenographic and thoracoscopic examinations do not disclose any abnormality. Although these instances are infrequent, this knowledge is of value in recurrent cases for it may permit one to avoid an unnecessary thoracotomy. Meade and Blades (13) encountered three patients among a group of eight operated upon for relapsing pneumothorax in whom no lesion could be found when the chest was opened. In many instances, blebs cannot be seen readily by roentgenography. This is especially true with

the small diffuse multiple disseminated emphysematous vesicles on the pulmonary cortex whose rupture is often responsible for spontaneous pneumothorax. Thoracoscopy is also valuable in properly evaluating the size of these lesions in considering the necessity for excisional surgery. Such complications as "pleural shock," hemorrhage, tension pneumothorax, or intrapleural infection associated with thoracoscopy did not occur in any patient in this series. Thoracoscopy can be performed easily, quickly, and painlessly and the procedure requires a minimum of equipment.

There appears to be a definite indication for the introduction of some foreign substance into the pleural space which will stimulate an inflammatory reaction and promote pleural symphysis. This has been achieved by the use of such substances as fresh whole blood, hypertonic glucose, kaolin, turpentine, and silver nitrate as well as talc (magnesium silicate). Silver nitrate was also used in this series and found to be effective, but the present writers have abandoned the use of this agent because it produces excruciating pain and frequently high fever. Concentrated glucose solution possesses similar undesirable properties. Talc is less irritating, but it produces adequate pleural symphysis. Bernard and Meyer (14) have reported a ten-year experience with talc poudrage, and during that period they encountered no instance of talc granuloma or other complication from its use. Open thoracotomy has been performed for other reasons on 2 of these patients shortly after talc poudrage was used and in each instance satisfactory pleural symphysis, comparable with that observed by other writers (5, 13), was found.

Hughes and associates (15) and others (16–18) have pointed out the advantages of removing the entrapped air by catheter suction, mentioning the rapidity of re-expansion and decreased period of patient disability. The success or failure of this treatment depends upon the closure of the ruptured bleb. The consistent manner in which these blebs become sealed once the entrapped air is removed and re-expansion achieved by active underwater seal suction is the crucial factor responsible for the high degree of success of this method of management.

Although it is undoubtly small, there is a certain hazard in the conservative management of spontaneous pneumothorax. For example, when one reviews the first roentgenograms of a patient with this condition and observes a collapse of, say, 50 per cent, it is not immediately obvious whether the collapse is increasing, decreasing, or stationary. An occasional patient will develop a tension pneumothorax while under medical observation. This accident can be obviated, of course, by performing a thoracentesis, taking intrapleural pressure measurements, removing some of the air, and repeating the measurements. This permits one to determine whether the leak is sealed or remains open. Without this precaution, however, one may leave such a patient with a small collapse, only to return shortly thereafter to find that a serious tension pneumothorax has developed. Furthermore, blebs temporarily sealed in a collapsed lung may reopen as the lung re-expands. With active suction after thoracoscopy and poudrage, these hazards do not exist.

Excisional pulmonary surgery was indicated in only 3 patients in this series. Open thoracotomy was performed because numerous large emphysematous blebs

were demonstrated in each of them; one of these patients had had repeated episodes of collapse. They were treated by simple excision of the blebs, vigorous scrubbing of the parietal pleura with dry gauze, and the addition of talc poudrage as a further stimulus to pleural symphysis. Open thoracotomy should be reserved for those patients who demonstrate large emphysematous blebs or bullae compressing healthy lung tissue, as demonstrated on chest roentgenograms and visualized by thoracoscopy.

Summary

An analysis of 80 cases of spontaneous pneumothorax is presented. Spontaneous pneumothorax should not be considered a completely benign condition, because it has resulted in death, and complications associated with it are not rare. Thoracoscopy as an aid in diagnosis and in selecting the treatment of choice is emphasized. The rupture of a subpleural bleb is the most common cause of "idiopathic" spontaneous pneumothorax.

A method of treatment for spontaneous pneumothorax which achieves pleural symphysis by talc poudrage and active underwater seal catheter suction is described. This method appreciably decreases the time required for re-expansion. The decreased period of disability among patients thus managed results in a great saving in money and manpower in comparison with other forms of therapy. Recurrent pneumothorax following talc poudrage has not occurred in the present experience, and it has been concluded that it is distinctly rare. Open thoracotomy is reserved for those patients who demonstrate large emphysematous blebs compressing healthy lung tissue. This is determined by roentgenographic and thoracoscopic examination of the chest.

Sumario

Le Assistencia del Neumotórax Espontáneo

Preséntase aquí un análisis de 80 casos de neumotórax espontáneo. No debe considerarse el neumotórax espontáneo como dolencia absolutamente benigna, pues ha acarreado la muerte y no son raras las complicaciones enlazadas con el mismo. Recálcase la toracoscopia como auxiliar en el diagnóstico y en la selección del tratamiento de elección. La rotura de una flictena subpleural constituye la causa más común del neumotórax espontáneo "idiopático."

Describese un método de tratamiento que obtiene la pleurodesis por el polvoreamiento con talco y la succión activa bajo agua con sonda sellada. Este método acorta decididamente el tiempo requerido para la reexpansión. El menor período de incapacidad entre los enfermos atendidos en esta forma da por resultado un gran ahorro de tiempo y de mano de obra, comparado con otras formas de terapéutica. No ha habido neumotórax recurrente consecutivamente al polvoreamiento con talco en la casuística de los AA., deduciéndose que es manifiestamente raro. La toracotomía a cielo abierto se reserva para los enfermos que muestran grandes flictenas enfisematosas que comprimen tejido pulmonar sano, lo cual se determina con el examen roentgenográfico y toracoscópico.

Resume

Le traitement du pneumothorax spontané

Présentation de l'analyse de 80 cas de pneumothorax spontané. Le pneumothorax spontané ne doit pas être considéré comme une lésion absolument bénigne, car il y a eu des

décès et les complications qui s'y associent ne sont pas rares. La thoracoscopie comme moyen de diagnostic et de sélection du traitement de choix est mise en valeur. La rupture d'une lésion bulleuse subpleurale est la cause la plus commune du pneumothorax spontané «idiopathique.»

L'auteur décrit une méthode de traitement permettant de réaliser la symphyse pleurale par le poudrage au talc et l'occlusion active sous courant d'eau par aspiration à la sonde. Cette méthode abrège de façon appréciable le temps requis pour la réexpansion. La réduction de la période d'invalidité chez les malades ainsi traités entraîne une grande économie d'argent et de main d'oeuvre, par comparaison avec les autres procédés thérapeutiques. Selon l'expérience de l'auteur, la reproduction du pneumothorax n'a pas été observée à la suite du poudrage au talc, et la conclusion est que ce fait est particulièrement rare. La thoracotomie «ouverte» est réservée aux patients présentant des vésicules emphysémateuses comprimant un parenchyme pulmonaire sain. Ces conditions sont déterminées par radiographie et thoracoscopie.

Addendum

The material on which this article is based was presented before a joint meeting of the Staff of the U. S. Naval Hospital, Portsmouth, Virginia, and the Norfolk County Medical Society, Norfolk-Portsmouth, Virginia, on April 19, 1954 (by A. G. M.). Since that time we have treated 27 additional patients with spontaneous pneumothorax by thoracoscopy, intrapleural talc poudrage, and active suction. Prompt re-expansion has been accomplished in every patient so treated. There have been no complications or sequelae associated with the procedure.

One patient was admitted recently with a 25 per cent collapse on the left which had occurred spontaneously. He had a small pleural effusion. Thoracoscopic examination revealed apical emphysematous blebs and the bleb which had ruptured was visualized. Talc poudrage was carried out, and the lung was promptly re-expanded by suction. Three days later, as he was about to return to duty, a spontaneous pneumothorax occurred on the right. This was treated in the same manner as had been employed on the left, and blebs were also visualized on the right side. Collapse on the right side was total, but immediate re-expansion was obtained and the catheter was removed forty-eight hours later. Three days following its removal, partial collapse of the right lung again occurred. The collapse amounted to approximately 25 per cent and was of a loculated type so that it was necessary to insert both an anterior and a posterior tube to secure re-expansion. It is obvious that this "relapse" occurred before sufficient time had elapsed to permit firm adhesions to form.

Emphysematous blebs were noted on thoracoscopic examination in 24 of these 27 additional patients. None of the 52 patients treated by intrapleural talc poudrage and active suction has returned to the hospital with recurrent pneumothorax. No patient has developed intrapleural infection or a significant pleural effusion after poudrage treatment. Hemothorax has not occurred.

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THE MECHANISM OF PNEUMOTHORAX INDUCTION[1]

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(Received for publication April 24, 1955)

INTRODUCTION

Although nineteen years have elapsed since Tchertkoff (1) reported that the usual technique for inducing pneumothorax creates a pleural space in almost every case *even if air is not injected into the chest*, the mechanism by which pneumothorax is induced continues to be disputed. Some writers (1–4) believe that the artificial creation of an intrapleural space with a needle is usually due to laceration of the visceral pleura so that air escapes from the lung. Others (5–7) believe that lung laceration seldom occurs at induction and that it is preventable by careful technique. The latter thesis is based on the assumption that it is possible to insert the point of a needle between the two layers of pleura without trauma to the lung, despite the fact that the force of hydraulic traction tends to keep the pleurae in contact.

Although the question involved in this controversy is seldom of clinical significance, it is certainly of theoretical interest. Therefore, the answer was sought by a direct approach. The purpose of this paper is to report the findings obtained on direct inspection of the pleural space and lung at thoracotomy following attempted induction of pneumothorax.

METHOD AND MATERIAL

Pneumothorax induction was attempted in 20 patients just prior to thoracotomy by the method described by Vysniauskas (7). With the patient under anesthesia, prior to incision of the chest wall, the skin of the superior aspect of the chest was punctured with an ordinary 18-gauge needle in order to remove a skin plug. Then a fresh 18-gauge needle, attached to a 2 cc. syringe filled with saline (8 cases) or methylene blue solution (12 cases), was inserted through the skin hole and advanced perpendicularly slowly with great caution through the intercostal tissues. As soon as the piston of the syringe was noted to descend, advance of the needle was halted and this position was maintained for several seconds before the needle was removed from the chest wall. If the piston did not descend, the needle was advanced only as deep as the thickness of the chest wall was thought to be.

Thoracotomy was performed in the usual manner. Subperiosteal rib resection was done with care in order to avoid nicking the parietal pleura. The proximity of the lung to the parietal pleura was then determined by inspection through the semitranslucent tissue. The pleural cavity was entered, and the lung was inspected for a puncture hole and for the stain of methylene blue. The presence of a puncture in the lung was demonstrated by flooding the lung surface with saline, expanding the lung by manual compression of the anesthesia bag, and noting the escape of bubbles at the puncture site if it existed. The pleural cavity was entered within less than thirty minutes from the time of attempted pneumothorax induction.

Most of the patients were young males, as would be expected in a military institution.

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TABLE 1

OPERATIVE FINDINGS IN 20 CASES OF PNEUMOTHORAX INDUCTION

Case Number	Diagnosis	Piston Sank	Adhesions	Space Produced	Lung Puncture	Location of Methylene Blue
1	Obstructive emphysema	no	none	no	no	
2	Coccidioidal cavity	?	none	no	no	
3	Histoplasmoma	yes	none	small	yes	
4	Atelectasis	no	few	no	no	
5	Tuberculosis	yes	none	no	no	
6	Metastatic cancer	yes	none	large	no	
7	Tuberculosis	?	none	no	no	
8	Coccidioidal cavity	no	none	large	yes	
9	Hamartoma	yes	none	small	yes	intrapleural
10	Histoplasmosis	yes	none	no	no	intrapleural
11	Tuberculosis	no	few	no	yes	intrapleural
12	Tuberculosis	no	few	no	?	intrapleural
13	Pulmonary sequestration	no	none	no	yes	intrapleural
14	Histoplasmoma	yes	none	?	no	intrapleural
15	Histoplasmoma	yes	none	no	no	intrapleural
16	Coccidioidal cavity	yes	none	small	no	intrapleural and beneath visceral peel
17	Tuberculosis	yes	few	no	yes	intrapleural
18	Bronchogenic cancer	yes	none	small	yes	intrapleural
19	"Tuberculoma"*	?	none	no	no	intrapleural
20	Diaphragmatic hernia	?	none	no	no	intrapleural

* Encapsulated caseous focus, etiology undetermined.

Only 5 patients had tuberculosis, and the indication for thoracotomy in these cases was therapeutic resection after many months of antimicrobial drugs. The indication was therapeutic for the patients with obstructive emphysema, coccidioidal cavity, and diaphragmatic hernia. In the remainder of the cases, thoracotomy was diagnostic as well as therapeutic.

RESULTS

The pertinent data in the series are presented in table 1. In 10 of the 20 cases the piston definitely descended in the barrel of the syringe and, in 5 of these 10 instances, a pneumothorax was noted prior to incision of the parietal pleura. The space was quite large in one patient. In only 3 of the 5 patients with pneumothorax was a puncture hole of the lung demonstrable. In one of the 10 cases in which the piston descended, the presence of a pneumothorax was uncertain and no puncture hole was demonstrated; in the remaining 4 cases the lung was seen to be in contact with the parietal pleura and yet a puncture hole was demonstrated in one case. Thus, a space and/or lung puncture was present in 6 of these 10 patients.

Among the 10 cases in which definite descent of the piston was not observed, movement of the piston was uncertain in 4 instances. In none of these was a pneumothorax present nor was a puncture of the lung demonstrated. In the remaining 6 cases of this group, a large pneumothorax was seen in one and

a puncture hole in 3, including the patient with the pneumothorax. Thus, a space and/or lung puncture was present in 3 of these 10 patients and in 9 of the total series of 20 patients.

In 12 patients methylene blue was used as the solution in the syringe, and the dye was found to be intrapleural in all cases. The piston sank in 7 cases and in 3 of these a pneumothorax was present. A puncture hole was demonstrated in 3 of the 7 patients: 2 with pneumothorax and one without. Pneumothorax was not present in any of the 5 cases in which the piston did not sink, but a puncture hole was demonstrable in 2 of them. Thus, a space and/or lung puncture was present in 6 of these 12 patients.

It is interesting to note that laceration of the lung was never linear but always punctate despite the fact that the anesthetized patient continued to breathe during induction of pneumothorax.

DISCUSSION

It appears that the truth lies somewhere between the two extremes of opinion on the subject of the mechanism of the pneumothorax induced for therapeutic purposes. Sometimes induction of pneumothorax is attended by puncture of the visceral pleura so that air escapes from the lung and creates an intrapleural space even though no air is introduced from without. Sometimes it is possible to insert a needle point between the two layers of pleura without traumatizing the lung. It is not possible, however, to predict which of these events will occur in any given case.

The present study further shows that one can induce pneumothorax and not be able to demonstrate a puncture of the lung on direct inspection under the conditions of this experiment. However, in such a case, it is necessary to postulate lung trauma anyhow since the intrapleural air, under the conditions of the experiment described herein, could come only from the pulmonary tissue. On the other hand, it is possible to puncture the lung and have no air escape into the pleural cavity, at least within thirty minutes. The data also show that one may puncture the lung and induce pneumothorax even when it is not known whether the needle has entered the pleural space preoperatively (as in those cases in which the syringe piston did not sink).

The unpredictability of the results in any particular patient subjected to initial pneumothorax suggests that uncontrollable factors influence the procedure. These include the patency of the needle as it passes through tissue, the elasticity and contractility of the lung, the strength of the visceral pleura in relation to the parietal pleura, as well as perhaps more subtle factors.

The presence of disease in the lung underlying the needle may be an important factor in the outcome of pneumothorax induction, since diseased lung may be more susceptible to laceration. From this standpoint the present series of cases is a selected one, as it was chiefly composed of young males with few or no pleural adhesions and highly localized pulmonary disease. Even the patients with tuberculosis were not of the type ordinarily considered for therapeutic pneumothorax. It is possible, therefore, that the incidence of lung trauma would

be higher if this experiment were to be done in a series of tuberculous patients for wh[illegible] pneumothorax would be indicated therapeutically. This statement seems reasonable because the disease would tend to be more active and more disseminated, so that the risk of lacerating lung tissue might be greater than in the present series of patients. This is a consideration which requires further study.

An additional factor to be considered in the interpretation of the results of the experiment is the time interval between attempted induction and inspection of the lung. This never exceeded thirty minutes, and this short interval may explain why some patients had evidence of lung trauma without pneumothorax. If the interval had been greater, air might have escaped slowly into the pleural cavity and the incidence of pneumothorax might have been higher.

In a previous publication (8) by one of the present writers (W. W.), it was reported that Tchertkoff's experiment was repeated in a small series of cases and his results were confirmed. The technique of induction was similar to that used in the present investigation, and no air was introduced into the chest from without. In 10 of the 15 cases, the syringe piston sank. All of the patients had tuberculosis for which pneumothorax was therapeutically indicated. Fluoroscopy, fifteen minutes and twenty-four hours after induction, revealed the presence of pneumothorax in 9 of the 10 patients, usually within fifteen minutes; but in several cases pneumothorax was not seen until twenty-four hours after induction.

In the present experiment the patients were under anesthesia and, therefore, did not have spontaneous marked increases in intrabronchial pressure which normally occur in the conscious person, as during talking, coughing, and similar actions. It was usually necessary to increase intrabronchial pressure by manual compression of the anesthesia bag in order to demonstrate a lung puncture by leakage of air under saline. The lack of normal increases in intrabronchial pressure in the anesthetized patients may be another explanation for the finding of a lung puncture occasionally in the absence of a pneumothorax within thirty minutes of induction.

In view of the above considerations, the incidence of lung trauma in the present series may represent the minimum to be expected. Nevertheless, escape of air from the lung and/or lung puncture was demonstrated in at least 6 of the 10 patients in whom there was preoperative evidence that the needle had entered the pleural cavity (sinking of the syringe piston) as well as in 3 of the 10 patients in whom such preoperative evidence was not obtained. Since great care was exercised during the procedure, it is apparent that lung trauma is common during induction of pneumothorax and is not preventable by this method. The manual dexterity of the physician should not be overrated.

The injection of air at the time of induction, as is usually done, may have virtue in that the resultant partial collapse of the lung may hasten sealing of the lung puncture which often results and may limit the escape of air from the traumatized lung. Under these conditions, an appreciable increment in the amount of intrapleural air over and above that which has been injected may be

difficult to detect except by gas analysis (4). This may explain some of the divergent results in the literature, but it does not prove that initiation of pneumothorax is seldom due to lung puncture.

The present experiment should be repeated with the use of other types of needles and methods of induction to determine whether lung trauma can be avoided. The size of the bevel of the needle may or may not be a factor which influences the results. It is possible for the relatively long bevel of an ordinary 18-gauge needle to lie partly within the pulmonary parenchyma and partly within the pleural cavity. However, Tchertkoff, Selikoff, and Robitzek (2) reported that their results were the same regardless of whether they used a sharp needle or a blunt one. It is also possible that lung puncture may occur less often if induction is done with a dry needle connected directly through rubber tubing to a water manometer because, as the needle first enters the pleural cavity, aspiration of air in the tubing might prevent laceration of the lung. These ideas are pure speculation, however, and it remains to be seen whether their validity will be confirmed by careful experimentation.

Initial pneumothorax is often traumatic to the underlying lung. Hence, it is easy to understand why it is difficult to obtain a space over atelectatic lungs (other factors may also be operative in this situation). The higher incidence of air embolism at initial pneumothorax than at refills, instances in which pneumothorax results even though intrapleural pressures cannot be obtained and air is not injected, and occasional excessive collapse of the lung despite the injection of only small amounts of air are some of the other factors.

SUMMARY

Pneumothorax induction was attempted in 20 patients and thoracotomy was performed immediately thereafter. Induction was performed with an ordinary needle attached to a fluid-filled syringe, and no air was introduced into the chest. Direct inspection of the pleural cavity and lung revealed that lung trauma is common. Analysis of the results indicates that lung trauma is not predictable nor is it preventable by the technique used in this investigation.

SUMARIO

El Mecanismo de la Inducción del Neumotórax

En 20 enfermos, se emprendió la inducción del neumotórax e inmediatamente después se ejecutó la toracotomía. La inducción se llevó a cabo con una aguja corriente unida a una jeringa llena de líquido, sin introducir aire en el tórax. La inspección directa de la cavidad pleural y del pulmón reveló que el traumatismo pulmonar era frecuente. El análisis de los resultados indica que no cabe predecir el traumatismo pulmonar y que tampoco es éste prevenible con la técnica usada en esta investigación.

RESUME

Le mécanisme de l'instauration du pneumothorax

La création d'un pneumothorax a été simulée chez 20 patients et une thoracotomie a été effectuée immédiatement. Pour cette expérience on avait utilisé une aiguille ordinaire fixée á une seringue remplie de liquide sans injection d'air dans le thorax. L'examen direct de la

cavité pleurale et du poumon a habituellement révélé un traumatisme du poumon. L'analyse des résultats indique que le traumatisme du poumon ne peut être ni prévu ni prévenu lorsqu'on emploie la technique utilisée dans cette étude.

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CHRONIC PULMONARY DISEASE IN HISTOPLASMIN REACTORS[1]

A Review of 229 Cases Discovered Through Chest Clinic Examinations

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(Received for publication February 7, 1955)

INTRODUCTION

As a result of studies completed during the past eight years, the former concept of histoplasmosis as a rare, almost universally fatal, generalized disease has been enlarged to include a benign condition of common occurrence and wide geographic distribution which may readily be confused with pulmonary tuberculosis.

In 1945, Christie and Peterson (1) and Palmer (2) demonstrated a relationship between nontuberculous pulmonary calcification and histoplasmin skin sensitivity and suggested that the calcifications might be the end stage of a nonfatal form of histoplasmosis. A similar and even more striking relationship has been shown by High and associates (3) in the case of disseminated pulmonary calcification which has been confirmed by White and Hill (4), who included observations of an antecedent pulmonary disease and suggested that the disease occurs in epidemic form. The suspicion that a nontuberculous pulmonary disease must precede the calcification was verified by the studies of Furcolow and his associates (5) and by Edwards and co-workers (6), who described pulmonary infiltration and hilar adenopathy associated with histoplasmin sensitivity in members of certain population groups. It is of interest that the latter writers, in a study of pulmonary infiltrates and antigen sensitivity among student nurses, reported that, of 224 nurses with pulmonary disease, 136 (65 per cent) reacted to histoplasmin and not to tuberculin. Likewise, as a result of the tuberculosis case-finding activities of the Ray Brook Hospital Clinic Service during the period 1946 to July, 1953, a total of 255 new cases of pulmonary tuberculosis were discovered, as well as 123 tuberculin-negative persons with chronic pulmonary disease presumed to be benign histoplasmosis. These data are not to be interpreted as a true index of the prevalence of histoplasmosis in the district. Some of the cases were recognized in retrospective studies and the sample may be limited to tuberculin-negative persons who, from the records, represent approximately one-half to one-third of the clinic population during the years studied.

The relatively frequent occurrence of histoplasmosis constitutes a diagnostic problem of increasingly important significance, with the widespread application of mass survey roentgenography to apparently healthy population groups. The present study was undertaken in an attempt to throw some light on the epidemiologic characteristics, the clinical and roentgenographic course, and the prognosis of a disease which is believed to be pulmonary histoplasmosis and is associated with histoplasmin skin sensitivity.

[1] From the Ray Brook State Tuberculosis Hospital, Ray Brook, New York.

MATERIALS AND METHODS

Since 1945 a number of cases of this hitherto unrecognized disease entity have been detected through diagnostic studies conducted by the Ray Brook State Tuberculosis Clinic Service and Hospital.[2] All of the persons included reacted to the histoplasmin skin test, and they have been divided into two groups on the basis of tuberculin sensitivity. In *Group A* are 123 persons who do not react to tuberculin, and in *Group B* there are 106 who react to tuberculin. In order to exclude acute lung disease, only those persons were included in the study in whom pulmonary infiltrates as demonstrated by serial chest films had been present for at least two months. Appropriate studies were carried out to exclude tuberculous and other chronic lung disease.

The Ray Brook Clinics are organized to provide diagnostic consultation service to the physicians of the district as an aid in tuberculosis control. New patients are drawn from three main groups in the population: household contacts of known cases of pulmonary tuberculosis; persons referred by their physician because of symptoms of chronic lung disease; and persons with abnormal chest films discovered through mass roentgenographic surveys. Clinic records since 1917 are available so that retrospective studies were possible in some instances.

Geographically, the clinic district may be divided into two main regions: a central mountainous area, comprising a portion of the Adirondack State Park, and two relatively level plains bordering on the St. Lawrence River and Lake Champlain. These are referred to hereafter as the mountain and valley regions, respectively. The mountain region is in large part forest land with villages and towns along the course of the mountain streams, while the valley region is an agricultural district with scattered villages, towns, and two small cities.

The clinic studies include a report of identification data and residence since birth, an occupational history, a history of contact with tuberculosis, a record of past and present illness, a physical examination frequently limited to the chest, tuberculin and histoplasmin skin tests, complement-fixation tests for histoplasmosis and blastomycosis, and postero-anterior, left lateral, and occasionally lordotic and oblique chest films. Regular clinics were held in the various population centers throughout the district and at the hospital. Whenever necessary and feasible, patients were hospitalized for differential diagnostic studies.

Old Tuberculin (OT) was given intradermally in 0.1 and 1.0 mg. dosage and histoplasmin as 0.1 ml. of an appropriate dilution.[3] Fresh dilutions of tuberculin were prepared every two weeks, and the histoplasmin at eight-week intervals. Separate syringes and needles were kept exclusively for the tuberculin and histoplasmin antigens. For both skin tests a reaction was considered positive if induration measuring 5 mm. or more in diameter was found at forty-eight hours. All of the persons in *Group A* failed to react to 0.1 mg. of OT, and in more than half of the persons no reaction was obtained with 1.0 mg. of this antigen. Complement-fixation tests for histoplasmosis and blastomycosis were made, using the yeastlike cell antigen.[4] Within the last three years the extract antigen of the yeastlike cell and the yeastlike cell antigen of *Blastomyces dermatitidis* have been employed. A reaction with a titer of 8 or greater was considered positive. A reaction titer less than 8 was considered to be of doubtful significance.

When available, specimens of sputum, gastric, and bronchial aspirations, pleural fluid,

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[2] The Ray Brook State Tuberculosis Clinics and Hospital serve the counties of Clinton, Essex, Franklin, Hamilton, and St. Lawrence in northeastern New York State which have a population of 238,649.

[3] Old Tuberculin was furnished by the New York State Department of Health, Division of Laboratories; and histoplasmin, standardized according to the method described by Howell, was obtained from the U. S. Public Health Service and Eli Lilly and Company.

[4] Complement-fixation tests were performed under the direction of Dr. Elizabeth Hazen of the New York State Department of Health.

TABLE 1

GROUP A CASES: HISTOPLASMIN-POSITIVE, TUBERCULIN-NEGATIVE
Age and Sex

Age	Male	Female	Total
Total	74	49	123
0–4 years	1	0	1
5–14 years	8	1	9
15–24 years	17	19	36
25–54 years	37	23	60
55 years and older	11	6	17

blood, bone marrow, lymph node and lung biopsy, in addition to material removed by pulmonary resection, were studied for tubercle bacilli and fungi by microscopy, culture, and animal inoculation. The details of these studies are described by Kurung (7). Animal inoculation was used occasionally early in the study. More recently, only culture examinations were performed. Tissue sections were prepared from pathologic material obtained by pulmonary resection or autopsy and were examined after staining with hematoxylin-eosin and periodic acid Schiff reaction.

OBSERVATIONS

The epidemiologic, clinical, and roentgenographic data given below were obtained from an analysis of 123 *Group A* cases in which failure to react to tuberculin would tend to exclude tuberculosis as a cause of the disease.

General Characteristics of the Group

Age and sex: At the time the lung disease was first recognized, the age of the patients ranged from three to seventy-six years. There were 74 males and 49 females. The age distribution by sex is shown in table 1. All were white.

Residence: For the purpose of the study, anyone who had resided in a region for two-thirds of a lifetime was assigned as a resident of that district. Of the 123 tuberculin nonreactors, 73 (59 per cent) were rural and 48 (39 per cent) were urban residents. In 2 instances residence was not determined. A total of 112 (91 per cent) resided in one or other of the valley regions; whereas, 5 were from the mountain and 6 were wanderers who had lived for various periods of time in the Ray Brook Hospital District and elsewhere, mainly in New York State or Canada.

Dust exposure: Exposure to certain dusts containing organic material has been suggested as a possible mode of infection in pulmonary histoplasmosis, especially in the epidemic form of the disease (4, 10–16). A history of exposure to dust was obtained in 67 of the 123 patients. The patients were questioned regarding exposure to dust near the habitat of domestic fowl or pigeons, to dust of threshing or filling silos, and to dust raised in handling raw wool or fleeces. No history of significant dust exposure was given by 14 patients (20 per cent). The remaining 53 (80 per cent) gave a history of definite dust exposure. Of these, 27 were exposed to dust near the habitat of domestic fowl or pigeons; 8, to dust of threshing

operations, filling silos, and handling raw wool; and 18, to a combination of two or three of the above exposures.

Occupation: A wide diversity of occupations was found in the group. The fact that 51 were farmers, farm laborers, lumbermen, rural housewives, or rural students is of particular interest. There were 16 urban students, 11 factory or mill workers, 8 urban housewives, 5 stock or grocery clerks, 5 common laborers, 4 office workers, 4 schoolteachers, 3 automobile mechanics, and 2 railroad workers. The following occupations were represented by one each: parish priest, roofer, telephone lineman, baker, customs inspector, hotel worker, telephone operator, civil engineer, truck driver, janitor, mill or plant owner, bartender, inspector, and retired factory worker.

Roentgenographic Classification

On the basis of the initial chest film, the cases were grouped into two main categories:

Disseminated pulmonary disease characterized by abnormal shadows which were widely and symmetrically scattered throughout both lung fields

Nondisseminated disease in which the abnormal shadows were frequently single, occasionally multiple, but were less widely and symmetrically distributed

The cases were further subdivided with respect to the mottled and nodular appearance of the shadows and the presence or absence of hilar adenopathy. The distribution of the cases under this classification is illustrated in table 2. Pleural effusion alone was noted in 2 cases and as a complication of the pulmonary disease in 7 additional cases, making a total of 9 cases for the group. Further observation demonstrated that, following the disappearance of the effusion in nearly every instance, some evidence of residual pleurisy was found. In one patient the sole manifestation of pulmonary disease was hilar enlargement.

Tuberculin Reactors

An epidemiologic and clinical analysis of the tuberculin reaction (*Group B* cases) was not made. Roentgenographically, the lesions were similar to those

TABLE 2

ROENTGENOGRAPHIC CLASSIFICATION AT TIME OF INITIAL OBSERVATION

Group A

	Total	Mottled	Mottled with Hilar Enlargement	Nodular	Nodular with Hilar Enlargement
Disseminated	39	9*	6	10	14
Nondisseminated	81	7*	16*	37*	21
Pleural effusion	2				
Hilar enlargement only	1				
Total	123	16	22	47	35

* Indicates number in each group with pleural effusion.

TABLE 3

LENGTH OF OBSERVATION

Years	Number of Patients
Less than 1	2
1–2	4
2–5	6
5–10	6
10–15	12
More than 15	4

Disseminated pulmonary disease was found in 16 and nondisseminated in 18 patients.

in *Group A* in that there were 64 persons with nondisseminated lesions and 42 with disseminated lung disease.

Cases with Established Date of Onset

The importance of the study is enhanced by the fact that, from both groups, 34 patients were seen in whom the illness had been observed from the onset. The majority were followed from the first symptom until pulmonary calcification developed. All of the patients in *Group B* included in the following analysis had one or more positive complement-fixation tests for histoplasmosis during the observed course of the disease except for one patient in whom *Histoplasma capsulatum* was isolated from the sputum. An analysis of these cases was undertaken to clarify the time relationship in the development of the disease from the clinical, roentgenographic, and epidemiologic aspects, particularly with respect to the early stages of the illness. In the 34 cases the length of observation varied from two months to twenty years and eight months. The distribution of the patients with respect to length of clinical observations is illustrated in table 3.

Source of the infection: The 34 patients were questioned about exposure to organic dust. No history of dust exposure was reported by 17 of the group. Eleven of the patients were from the Plattsburg epidemic of histoplasmosis (8). These patients while engaged in the demolition of a school building in Plattsburg, New York, had been exposed to the dust near the habitat of pigeons. A history of exposure to dust near the habitat of domestic fowl was given by 3 persons. Exposure to the dust of threshing, to dust near silo-filling operations, and to dust

TABLE 4

INCUBATION PERIOD

Days	Number of Patients
7	3
8–10	6
11–14	4
21	1
Unknown	20
Total	34

in a cellar while moving coal occurred shortly before symptoms of pulmonary disease appeared in 3 others. The total of those who gave some history of dust exposure was 17 persons. It is of interest that all who gave a history of dust exposure had disseminated pulmonary disease.

Incubation period: Information about the time interval between exposure to organic dust and the development of symptoms was obtained in 14 of the 34 cases. Eleven of these were from the Plattsburg epidemic. The distribution in days of the incubation period is illustrated in table 4.

Clinical Manifestations

In general, the symptoms in the nondisseminated disease were mild while, in the disseminated disease, they were moderately severe. For this reason the symptoms were enumerated according to roentgenographic classification and further subdivided into initial (those occurring during the first six weeks of the illness) and persistent (those which occurred thereafter).

Nondisseminated disease

Initial symptoms: Among the 18 patients in this category, no symptoms were reported by 3 of them. In the order of the frequency of occurrence, the symptoms recorded for the remaining 15 patients were: cough, lassitude, chest pains, expectoration, fever, weight loss, hemoptysis, dyspnea, wheezing, chills, anorexia, and aching pain throughout the body. The cough when first noted was dry. Expectoration of thick, tenacious, whitish or yellow sputum usually appeared after four to seven days.

Persistent symptoms: All symptoms disappeared in 10 of the 18 patients after six weeks and did not return. Among the remainder, lassitude, slight cough, expectoration, night sweats, weight loss, chest pain, and hemoptysis were found in the order of decreasing frequency of occurrence.

Disseminated disease

Initial symptoms: In 2 of the 16 patients with disseminated lung disease, no complaints were noted during the first six weeks of the illness. Among the remaining 14 the symptoms were moderate to severe and in the order of frequency of occurrence included: cough, chills, fever, expectoration, malaise, lassitude, weight loss, headache, chest pain, anorexia, nausea, vomiting, abnormal sweating, aching pain throughout the body, dyspnea, dizziness, diarrhea, constipation, chest wheezing, epigastric distress, and a generalized pustular eruption. As was noted in the nondisseminated disease, cough which at first was nonproductive was followed by expectoration of a thick, tenacious, white or yellow sputum after four to seven days.

Persistent symptoms: In the order of frequency of occurrence, the symptoms noted after six weeks from the onset of the disease and thereafter in 14 of the patients with disseminated lung disease were: dyspnea, lassitude, cough, expectoration, chest pain, night sweats, joint pain, and hemoptysis.

Physical signs

Initial: A paucity of abnormal physical signs characterized both the disseminated and the nondisseminated form of pulmonary histoplasmosis. For this reason, the physical signs recorded for the 34 cases are enumerated together. The initial physical signs are unknown in 19 of the 34 cases, as a physical examination was not made during the first six weeks of the illness. No abnormalities were found in 9 of the 15 patients examined. The physical abnormalities recorded were: rales in all 6 patients, rhonchi in 2, and a friction rub in one patient.

Persistent: Persistent signs were not found in 27 patients examined repeatedly after the first six weeks of the illness. Rales were recorded in 4 patients, rhonchi in 2, and breath sounds were diminished and could not be voluntarily intensified in one patient.

Roentgenographic Course

A detailed analysis was made of the chest films which were taken of 34 patients with histoplasmosis in order that information might be obtained of the roentgenographic course of the disease. The chest films were studied from the onset to the stage of pulmonary calcification, with special reference to the time factors involved in the changing chest films. In the interest of clarity, it is suggested that attention be focused on a single abnormal shadow and the changes be described which occurred in that shadow as the disease progressed.

> In both the disseminated and nondisseminated forms of the disease, the infiltrate at the onset appeared irregularly mottled. After a period of time, which might be as short as one month or as long as twenty months, the mottled shadows shrank and became nodular. However, in 7 of the 34 patients, mottled shadows persisted longer than two years. The nodular densities gradually shrank and became spherical or ovoid. Evidence of beginning calcification usually appeared in the center of the nodule after a period of three to fifteen years. Characteristically, the central calcific density did not increase in size, but the less dense shadow surrounding it gradually cleared over a period which varied from six to more than thirteen years. Occasionally, the nodular density did not change in size, but an area of calcification of the same size as the noncalcific density ensued. When roentgenographic evidence of hilar adenopathy was noted, it usually cleared within two to four months, but occasionally hilar adenopathy has persisted as long as five years.

Hilar calcifications have been demonstrated three and one-half years after the onset of the the disease, but the appearance of these calcifications may be delayed as long as ten years.

Follow-Up of the Plattsburg Epidemic

Additional data have accumulated with respect to the status of 10 patients from the Plattsburg epidemic, originally studied by Nauen and Korns (8) and previously reported by the present writer (4). The present status of these cases is recorded graphically in table 5. It is of interest that *Case 11* is that of a man who worked in the partially demolished school building four months after the epidemic and who, approximately one week later, became ill with fatigue, weak-

TABLE 5

FOLLOW-UP OBSERVATIONS ON 11 PATIENTS OF THE PLATTSBURG EPIDEMIC

Case Number	Tuberculin Skin Test	Histoplasmin Skin Test	Complement-Fixation Test for Histoplasmosis	Last Date of Observation	Persistent Symptoms
1. G. B.	Positive	Positive	9-14-49 Negative 9-12-51 Negative 2-10-54 Negative	2-10-54	Chest pain, mild lassitude
2. P. C.	Negative	Positive	1-14-49 Titer 35	6-16-49	Dyspnea
3. E. D.	Positive	Positive	1-14-49 Titer 27	1-14-49	None
4. O. F.	Positive	Positive	1-14-19 Titer 15 7-11-51 Questionable 11-10-54 Questionable	11-10-54	Slight cough with expectoration and lassitude
5. A. H.	Positive	Positive	1-14-49 Titer 18 9-14-53 Negative	9-14-53	Slight cough and expectoration, slight dyspnea and lassitude
6. S. M.	Negative	Positive	1-14-49 Titer 10 11- 9-49 Negative 9-15-54 Negative	9-15-54	Dyspnea and easy fatigability
7. G. M.	Positive	Positive	1-14-49 Titer 13 10- 6-54 Negative	10-31-54	Slight dyspnea, easy fatigability, cough, expectoration, intermittent chest pain, and wheezing
8. W. S.	Positive	Positive	12-13-50 Negative 3-11-53 Negative 4-13-54 Negative	4-13-54	Moderate to severe dyspnea, worse at time of colds and on exposure to dust. Occasional aching pain in both anterior chests
9. W. W.	Positive	Positive	3- 7-49 Titer 70 5-24-50 Titer 20 5-14-52 Questionable 10-14-53 Negative 11-10-54 Questionable	11-10-54	Lassitude and dyspnea
10. W. P.	Positive	Positive	3- 7-49 Titer 150 5-11-54 Atypical	5-11-54	Dyspnea
11. H. D.	Positive	Positive	1-14-49 Titer 34	1-14-49	Dyspnea and lassitude

ness, chills, fever, pain in the back and legs, cough, and expectoration. He was ill in bed for three weeks. A chest roentgenogram revealed disseminated mottled shadows in both lungs. Follow-up examination has revealed that this man is still suffering from slight dyspnea and lassitude. The follow-up observations for the group as a whole may be seen in table 5.

Prognosis

The disease as observed in 229 cases of pulmonary histoplasmosis is usually a mild self-limiting infection in which the prognosis is excellent. No deaths have occurred during the period of observation. Although a number of persons with disseminated disease, notably those of the Plattsburg epidemic, suffered a moderately severe illness initially, recovery was the rule without marked interference with normal working capacity. In one patient the clinical course was characterized by exacerbations and remissions of symptoms which were sufficiently severe that hospitalization was necessary on three occasions during a ten-year period.

Relapse

One or more roentgenographic changes indicating a relapse or an unfavorable course of the disease were seen in 7 of the 229 patients. In 4 instances new mottled shadows appeared in the chest film of a person in whom evidence of pulmonary disease, presumably due to histoplasmosis, pre-existed.

The first of these was the case of a boy eight years of age who was negative to tuberculin, positive to histoplasmin, and who had partially calcified pulmonary and hilar disease on the left. Subsequently he developed mottled shadows in the right mid-lung with enlargement of the right hilum and a positive complement-fixation test for histoplasmosis in a titer of 100. The titer gradually fell to 25 during a three-month period, with partial clearing of the shadows in the chest film.

The second case was that of a fifty-six-year-old housewife in whom nodular densities in the left upper lobe were first seen in October, 1951. The tuberculin skin test was negative. The histoplasmin skin test was positive. In May, 1953, she developed a large nodular density in the left lower lobe which measured 4 cm. in diameter. By July, 1953, the nodule had increased in size to 4.5 cm. in diameter and by September, 1953, evidence of cavity formation was noted in the center of the nodule.

The third case was that of a twenty-seven-year old housewife who was found with evidence of scattered disease over the upper third of both lungs in July, 1937. While still under observation in July, 1945, at the time of an increase in symptoms, roentgenographic evidence of new disease appeared in the right third and fourth anterior interspaces. The roentgenographic evidence of the original and the new disease persisted and eventually went on to calcification.

The fourth case had previously been reported by Monroe and Kurung (9). When the patient was first seen in July, 1939, he had right pulmonary and hilar disease and a bilateral pleural effusion. The shadows subsequently cleared almost

completely and the patient apparently remained well until June, 1951, when after a three-month illness a chest film revealed bilateral upper lobe pulmonary disease with bilateral cavity formation. *Histoplasma capsulatum* was repeatedly cultured from the sputum of this patient.

In addition to the 2 cases described above with roentgenographic evidence of pulmonary cavity, 2 other patients were seen in whom nodular densities apparently excavated, producing pulmonary cavities.

The seventh case was that of a person with bilateral nodular pulmonary disease in whom three large nodular densities were seen on the right and seven on the left. While under observation for a period of two years, central or so-called "halo calcification" was noted in several of the nodules bilaterally. Between January, 1947, and September, 1951, a definite increase in the size of one nodule on the right and two on the left occurred and persisted in subsequent examinations without further increase in size and without symptoms.

Microbiologic Studies

During the period of the present study, methods have been developed at the Ray Brook State Tuberculosis Hospital for the isolation of *Histoplasma capsulatum* from infected material (7). Sputum was obtained for examination for tubercle bacilli from 120 of the 229 patients; and a total of 443 examinations were performed by microscopy alone in 39 instances, by microscopy and culture in 397 instances, and by guinea pig inoculation in 13 cases. All were negative for tubercle bacilli.

Sputum specimens for examination for fungi were submitted by 46 patients. A total of 150 specimens were examined by microscopy and culture and by guinea pig inoculation in 3 instances. Organisms morphologically identical with *Histoplasma capsulatum* were seen in two consecutive freshly collected specimens of sputum obtained during the month of September, 1947, from one patient. The significance of this finding is not clear, as *Histoplasma capsulatum* was not isolated by culture from these specimens. *Histoplasma capsulatum* was cultured from six specimens and isolated on guinea pig inoculation from one specimen, all from a single patient whose case had previously been reported by Monroe and Kurung (9). All other sputum studies for fungi were negative.

The following miscellaneous examinations were performed: bronchial aspirations were examined for tubercle bacilli and fungi in 3 patients; aural discharges were studied for tuberculosis and fungi in 2 patients; blood specimens of 2 patients and bone marrow of one patient were examined for tubercle bacilli and fungi. Pleural fluid of one patient was cultured for tubercle bacilli. No organisms were found by any of these examinations.

Pulmonary resections of nodular lesions were performed on 3 patients from the tuberculin-positive group. Cultural examinations for fungi and culture and guinea pig inoculations for tubercle bacilli were negative. Organisms morphologically identical with *Histoplasma capsulatum* were seen in tissue sections prepared from the resected specimen of one patient. Coccidioidin skin tests were performed on 6 patients and were negative.

TABLE 6

COMPLEMENT-FIXATION TESTS FOR HISTOPLASMOSIS BY DATE AND REACTION TITER

Case Number	Age	Sex	Roentgenographic Classification	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth
					Group A							
1. H. B.	12	M	Nondisseminated nodular	1-8-51 Titer 30	9-19-52 Titer 8							
2. L. B.	8	M	Nondisseminated nodular	8-31-50 Titer 28	8-24-51 Negative							
3. P. C.	24	M	Nondisseminated nodular	1-14-49 Titer 35								
4. R. D.	16	M	Pleural effusion only	12-5-50 Titer 20								
5. J. D.	16	M	Disseminated mottled	10-5-49 Titer 100	10-31-49 Titer 70	11-8-49 Titer 60	2-6-50 Titer 70	5-1-50 Titer 40	7-5-50 Titer 30	11-21-50 Titer 25		
6. E. D.	33	F	Nondisseminated nodular	4-5-50 Titer 50	7-5-50 Titer 45	11-5-51 Atypical						
7. J. F.	18	M	Disseminated mottled	3-22-50 Titer 30	7-3-51 Negative							
8. I. K.	33	M	Disseminated mottled	4-28-48 Titer 32	5-1-48* Positive	7-6-48* Positive	6-12-50 Negative	4-7-52 Negative	5-11-53 Negative			
9. T. L.	7	M	Nondisseminated mottled	4-4-50 Titer 80	11-7-50 Titer 100	12-5-50 Titer 100	7-3-51 Titer 50					
10. J. L.	16	M	Nondisseminated nodular	9-12-50 Titer 30	12-20-50 Titer 30							
11. P. L.	28	M	Disseminated nodular	10-11-49 Titer 80	2-13-50 Titer 60	4-13-54 Questionable						
12. M. L.	21	F	Nondisseminated nodular	1-12-49 Titer 20	6-10-52 Negative	9-14-53 Negative						
13. P. M.	13	M	Disseminated nodular, bilateral hilar	1-23-52 Titer 38	6-16-52 Atypical	5-19-54 Negative						

14. S. M.	39	M	Disseminated nodular	1-14-49 Titer 10	11-9-49 Negative	9-15-54 Negative					
15. M. M.	20	F	Disseminated nodular	3-9-49 Titer 20							
16. N. P.	27	M	Disseminated nodular	2-22-51 Titer 15	5-11-53 Titer 3	12-7-53 Negative					
17. C. P.	28	F	Nondissemi-nated mottled	6-9-48 Titer 16	1-4-49 Titer 30	9-8-49 Titer 20	11-10-49 Titer 30	1-22-51 Titer 25	12-29-53 Negative		
					Group B						
18. A. Mc.	43	F	Nondissemi-nated mottled	4-24-50 Titer 30							
19. A. G.	64	M	Disseminated nodular	1-11-50 Titer 30							
20. W. D.	57	M	Nondissemi-nated nodular	11-27-50 Titer 30							
21. K. H.	32	M	Disseminated nodular, calci-fied	1-4-51 Titer 15							
22. G. R.	28	M	Disseminated nodular	7-12-49 Titer 30	12-13-49 Titer 20	12-11-50 Titer 20	12-10-51 Negative				
23. M. N.	25	F	Nondissemi-nated mottled	1-4-51 Titer 30	4-12-51 Titer 8	6-19-51 Negative					
24. A. V.	27	M	Nondissemi-nated nodular	8-8-50 Titer 100	9-12-50 Titer 60	12-12-50 Titer 70	3-13-51 Titer 100	12-11-51 Titer 27	3-11-52 Atypical	5-13-52 Atypical	7-8-52 Atypical	8-11-53 Titer 6
25. H. D.	56	M	Nondissemi-nated nodular	1-14-49 Titer 34							
26. E. D.	37	M	Disseminated nodular, calci-fied	1-14-49 Titer 27							
27. O. F.	60	M	Disseminated nodular, par-tially calci-fied	1-14-49 Titer 15	7-11-51 Titer 8	11-10-54 Atypical					

TABLE 6—*Concluded*

Case Number	Age	Sex	Roentgenographic Classification	First	Second	Third	Fourth	Fifth	Sixth	Seventh	Eighth	Ninth
					Group B—Continued							
28. A. H.	45	M	Disseminated nodular, calcified	1-14-49 Titer 20	9-14-53 Negative							
29. G. M.	48	M	Disseminated nodular, calcified	1-14-49 Titer 13	10-6-54 Negative							
30. W. W.	33	M	Disseminated nodular, calcified	3-7-49 Titer 70	5-10-50 Titer 20	5-14-52 Atypical	10-14-53 Negative	11-10-54 Atypical				
31. W. P.	64	M	Disseminated nodular, calcified	3-7-49 Titer 150	5-11-54 Atypical							
32. D. W.	18	M	Nondisseminated mottled, pleural effusion, left	6-17-52 Titer 28	6-26-52 Titer 31	8-13-52 Titer 19	2-5-53 Titer 8	3-16-53 Atypical				
33. F. L.	34	F	Nondisseminated mottled	12-17-49 Negative	1-21-50 Titer 8	2-7-50 Titer 20	3-13-50 Titer 8	4-5-52 Titer 5	6-15-52 Negative	7-11-52 Negative		
34. I. M.	50	M	Disseminated nodular, calcified	6-10-49 Titer 100	6-16-52 Titer 100							

* Tests performed through the courtesy of Dr. M. L. Furcolow.

Complement-fixation tests for histoplasmosis were made on blood drawn from 172 of the 229 patients. A total of 292 tests were performed. Of these, single or multiple tests were reported as negative in 105 patients. In 32 persons a single test or one or more of several were reported as showing a slight reaction of doubtful significance or atypical reactions. In most cases, titers of less than 8 were obtained in these patients. Positive complement-fixation tests were reported in 34 persons, 11 of whom had single positive tests and 23 of whom had one or more positive reactions noted in serial tests with changing titers.

In table 6, the roentgenographic classification at the time of the first positive test, the age, and sex are correlated with the reaction titer of the complement-fixation test for histoplasmosis and the dates of these reactions. Although in the later years of the study the complement-fixation test was performed with both the yeast cell antigen of *Histoplasma capsulatum* and the extract antigen of the yeast cell, for the purpose of the table, only the reaction to the yeast cell antigen is reported. A complement-fixation test using the yeast cell antigen of *Blastomyces dermatitidis* was performed concurrently since 1951 and was reported as positive in a single patient. In that case the titer was lower for the heterologous antigen.

It is of interest that all of the patients in *Group A* with positive complement-fixation tests for histoplasmosis were less than forty years of age and all but 2 were less than thirty. In *Group B*, the older segment of the population is represented, due in part to the fact that 7 of these were from the Plattsburg epidemic which occurred in 1938. Among the 34 cases, mottled shadows were found in the chest films of 9 of them. In the experience of the writer, positive complement-fixation tests were most apt to be found when the shadows in the chest film were irregularly mottled, indicating recently acute disease. Nodular disease without calcification was noted in 16 persons, and a pleural effusion alone was seen in one instance. The remaining 8 positive tests occurred in persons with extensive disseminated disease in whom there was evidence of beginning calcification. Seven of the 8 were from the Plattsburg epidemic, with disease which had existed for eleven years before the test was done. In the eighth subject, disease which was similar roentgenographically to that of the other 7 had been present for thirteen years.

DISCUSSION

The above report records for the first time observations for a decade or more on the clinical course of a group of patients with a chronic lung disease believed to be benign histoplasmosis. The disease pattern varies from a mild subclinical infection associated with minimal pulmonary infiltration to a moderately severe nonfatal illness with disseminated lung disease. No case of severe generalized histoplasmosis has been observed.

It is noted in histoplasmosis, as in tuberculosis and coccidioidomycosis, that the specific skin test denotes previous infection with the causal organism but gives no clue to the presence or absence of activity of the infection. A positive histoplasmin skin test is frequently found without demonstrable evidence of disease in the lungs or elsewhere in the body. In previous studies (5, 6, 17) of

pulmonary infiltrates associated with histoplasmin sensitivity, children or young adult groups were represented; while in the present study all age groups are found. More than half of the patients in *Group A* were twenty-five years of age or older.

It is not clear whether the relatively large number of patients in the older age group is related to the fact that, in the region studied, where there is a relatively low prevalence of histoplasmin skin sensitivity in the population (4), the initial infection is delayed till later in life. The male preponderance in the present series in the ratio of 3 males to 2 females parallels the finding in histoplasmin skin sensitivity rates reported by others (18, 19); and the predilection of the disease to rural residents of valley regions has previously been noted (4, 20).

Evidence has accumulated that histoplasmosis like coccidiodomycosis may be an airborne infection which spreads from a natural reservoir of the fungus in the soil. Emmons (21) was the first of a number of investigators (22–26) to isolate *Histoplasma capsulatum* from the soil and to demonstrate that the fungus may be grown artificially in the laboratory on soil containing humus. Furcolow and Grayston (27) and Loosli and his associates (11, 12), in describing epidemics of histoplasmosis, were able to isolate *Histoplasma capsulatum* from the soil at or near the point source of the epidemic in eleven of thirteen epidemics studied by the former writers and in one epidemic in which careful clinical and epidemiologic studies were carried out by the latter group. It is of interest that nearly all of the epidemic cases of histoplasmosis were in persons with so-called disseminated pulmonary disease and that in these cases, as well as in a small number described in the present study, a definite relationship between pulmonary disease and exposure to organic dust has been established. The exact method by which man acquires the usually asymptomatic nondisseminated form of pulmonary histoplasmosis has not been shown.

The roentgenographic course of the disease is not characteristic and it cannot be distinguished from tuberculosis or other chronic lung infections on the basis of the chest film alone. A possible exception to this statement is the fact that cases of disseminated pulmonary disease which go on to disseminated pulmonary calcification, especially when they occur in regions where histoplasmosis is endemic, can usually be attributed to infection with *Histoplasma capsulatum*. The symptoms of both the disseminated and nondisseminated pulmonary disease closely resemble the ubiquitous acute respiratory infections, and for this reason a diagnosis of histoplasmosis is not suspected from symptoms alone unless a chest roentgenogram reveals evidence of chronic lung disease. It is noteworthy that benign pulmonary histoplasmosis was not generally recognized as a disease entity until mass roentgenographic and tuberculin-testing surveys were applied to healthy population groups.

Evidence gathered from experience with accidental laboratory infection with *Histoplasma capsulatum* (28) indicates that, although infection tends to occur among newly exposed histoplasmin-negative persons, previously infected persons have not had a second illness. In the present study it is apparent that, in a few persons, clinically inactive infection has shown roentgenographic and clinical

evidence of exacerbation. Jones (43) has reported one such case. Whether these represent instances of exogenous or endogenous reinfection is not clear.

Although it has been possible to establish an etiologic diagnosis in cases of severe generalized histoplasmosis once the mycotic etiology has been suspected (29), the discovery of the causal organisms in the milder infections which are usually localized in the lungs has been extremely difficult (30–33). For example, French and associates (34), in describing a case of histoplasmosis, record that, of forty cultures of sputum, gastric aspirations, or biopsy specimens, only one yielded a growth of *Histoplasma capsulatum*. The present study suggests that, with currently used mycologic techniques, it is unlikely that an etiologic diagnosis will be made in any significant number of cases of so-called benign histoplasmosis. Undiagnosed, these cases present a serious problem in management.

Serologic tests have been introduced as an aid in the diagnosis of histoplasmosis. Such tests have proved of value in other systemic mycoses, notably in coccidioidomycosis (35) and blastomycosis (36). In histoplasmosis a relationship has been established between infection with *Histoplasma capsulatum* in humans and the complement-fixation test for histoplasmosis (37–40). High titers have been found to be associated with active infection (39). Nevertheless, in cases of advanced histoplasmosis associated with severe endocrinopathy, complement-fixation tests have been reported as positive with low titers and, in addition, a number of proved cases of chronic histoplasmosis have been seen in which no detectable complement-fixing antibodies were found (40). One such case is referred to in the present study.

Cross-reactions have been reported mainly with *Blastomyces dermatitidis* and, to a lesser extent, with *Coccidioides immitis* (40–42). This drawback can be overcome by concurrent testing with blastomyces antigen. Significant cross-reactions have not been seen in the patients of the present study. Campbell and Binkley (40) also state that, in patients with "benign pulmonary self-limiting infections," the complement-fixing antibodies may appear early in the course of the disease, with a peak of antigenic titer during the first six weeks of the illness. The high level persists for a short time but by the fourth month the antibody titer has fallen to 1:20 or less. The findings in a number of cases in the present study are similar. Nevertheless, from table 4 it may be seen that titers higher than 20 may persist for a time considerably longer than four months in a significant number of cases. It is of interest, that in nine of the cases from the Plattsburg epidemic and in one additional case, complement-fixation tests were positive in titers which varied from 10 to 150 more than ten years after the onset of the disease. It should be emphasized that, when the tests were done, these patients were not clinically ill and that nearly all had been well and working since recovering from the acute epidemic illness. It appears probable that these may be chronic, mild, smoldering infections which may persist for a considerable period of time without severely incapacitating the patient. The present observations show that in these cases the complement-fixation test becomes negative at the time or soon after calcification of the pulmonary disease is complete.

Although *Histoplasma capsulatum* has not been recovered in the majority of

the cases included in the present study, the following points support the diagnosis of pulmonary histoplasmosis:

Observations have been made over a considerable number of years of a characteristic disease pattern in histoplasmin reactors. The pulmonary disease has varied in extent and distribution of lung involvement, but the course of the individual areas of disease from the early mottled infiltrate to pulmonary calcification has been strikingly similar, suggesting a uniform etiology.

Differential diagnostic studies have excluded other causes.

A positive serologic reaction for histoplasmosis has been found in a significant number of cases.

Important epidemiologic evidence was furnished by follow-up studies of the cases of the Plattsburg epidemic and has been supplemented by the isolation of *Histoplasma capsulatum* from the soil in Plattsburg at a point near the source of the epidemic (10).

The etiologic agent has been found in 3 of the cases reported.

In the tuberculin-positive patients of *Group B*, the failure after intensive search to isolate the tubercule bacillus does not completely exclude tuberculosis. Tubercle bacilli cannot be found in a small number of patients with minimal pulmonary tuberculosis. Histoplasmosis and tuberculosis have been reported to coexist (29).

The patient who reacts to both tuberculin and histoplasmin presents a difficult problem in management. It seems advisable to admit these patients to the hospital for a brief intensive study and to continue the observations through the outpatient department. Information gained from such study over a considerable period of time has strengthened the belief that these individuals have histoplasmosis rather than tuberculosis. The conclusion is supported by the study of patients in whom relapse has occurred and from whom tubercle bacilli were not isolated, in spite of roentgenographic evidence of active disease or pulmonary cavitation. Positive serologic tests for histoplasmosis have aided in the diagnosis, especially when a changing titer is found in serial tests.

The figures at the end of the article depict the roentgenographic and clinical features of typical cases of the disease.

SUMMARY

Two hundred and twenty-nine cases of a chronic pulmonary disease in histoplasmin reactors have been collected and observed in a tuberculosis chest clinic and hospital. The observation period in the majority of patients has varied from five to twenty years.

The disease was found among all age groups, predominantly in the males. A significant relationship has been shown between exposure to certain dusts containing organic material and the development of the disease. Although the condition occurs in persons engaged in a wide diversity of occupations, farmers and their families and rural residents are most likely to be affected. A roentgenographic classification based on the appearance of the abnormalities at the time

the patient was first seen is suggested. In 34 patients in whom the early stage of the disease was observed, epidemiologic and clinical information is presented.

A follow-up study of 11 cases from the Plattsburg epidemic is included.

A review of the clinical course of the disease with the roentgenographic, mycologic, and serologic findings has been presented and illustrated by typical cases.

SUMARIO

Enfermedad Pulmonar Crónica en los Reactores a la Histoplasmina: Presentación de 229 Casos Descubiertos por Conducto de una Clínica Torácica

Doscientos veintinueve casos de enfermedad pulmonar crónica fueron recogidos y observados en una clínica y hospital para tuberculosis del tórax. En la mayoría de los enfermos, el período de observación ha variado de cinco a veinte años.

La dolencia fué descubierta en todos los grupos de edades, predominando en los varones. Observóse una relación significativa entre la exposición a ciertos polvos que contenían materias orgánicas y la aparición de la enfermedad. Aunque la afección aparece en individuos dedicados a ocupaciones muy diversas, los labradores y sus familias y los residentes en el campo son los más propensos a afectarse. Propónese una clasificación roentgenográfica basada en el aspecto de la radiografía al observar al enfermo por primera vez.

Preséntase información epidemiológica y clínica para 34 enfermos en quienes se observó la etapa incipiente de la dolencia. Agrégase un estudio subsiguiente de 11 casos procedentes de la epidemia de Plattsburg. Preséntase e ilústrase con casos típicos una reseña de la evolución clínica de la enfermedad, con los hallazgos radiográficos, micológicos y serológicos.

RESUME

Maladie pulmonaire chronique chez des sujets ayant une réaction positive à l'histoplasmine: Rapport sur 229 cas découverts dans une clinique des voies respiratoires

Deux cent vingt-neuf cas d'affection chronique du poumon ont été réunis et observés dans un hôpital et une clinique des voies respiratoires. Dans la majorité des cas, la période d'observation du malade a varié entre cinq et vingt ans. La maladie a été observée parmi tous les groupes d'âge, elle prédominait chez l'homme. Une concordance significative a été démontrée entre l'exposition à certaines poussières renfermant des matières organiques et le développement de l'affection. Bien que cette maladie se produise chez des sujets ayant des occupations très diverses, les fermiers et leur famille ainsi que les habitants des campagnes sont parmi les plus souvent affectés. Une classification radiologique, fondée sur l'aspect du radiogramme au moment du premier examen du patient, est suggérée.

L'auteur présente des informations épidémiologiques et cliniques concernant 34 patients chez lesquels la phase initiale de l'affection a été observée. Une étude de 11 cas de l'épidémie de Plattsburg, qui ont été suivis, est incluse. Un compte-rendu du cours clinique de l'affection et des résultats des examens radiologiques, mycologiques et sérologiques est présenté et illustré par des observations de cas spécifiques.

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PLATE I

FIG. 1. Chest film of an 8-year-old boy (L. B.) taken two weeks after he became ill with fever, malaise, loose cough, and expectoration. All symptoms disappeared after eight weeks and he remained well. The histoplasmin test was positive; the tuberculin test, negative. Serologic test (CF) for histoplasmosis was positive, with a falling titer.

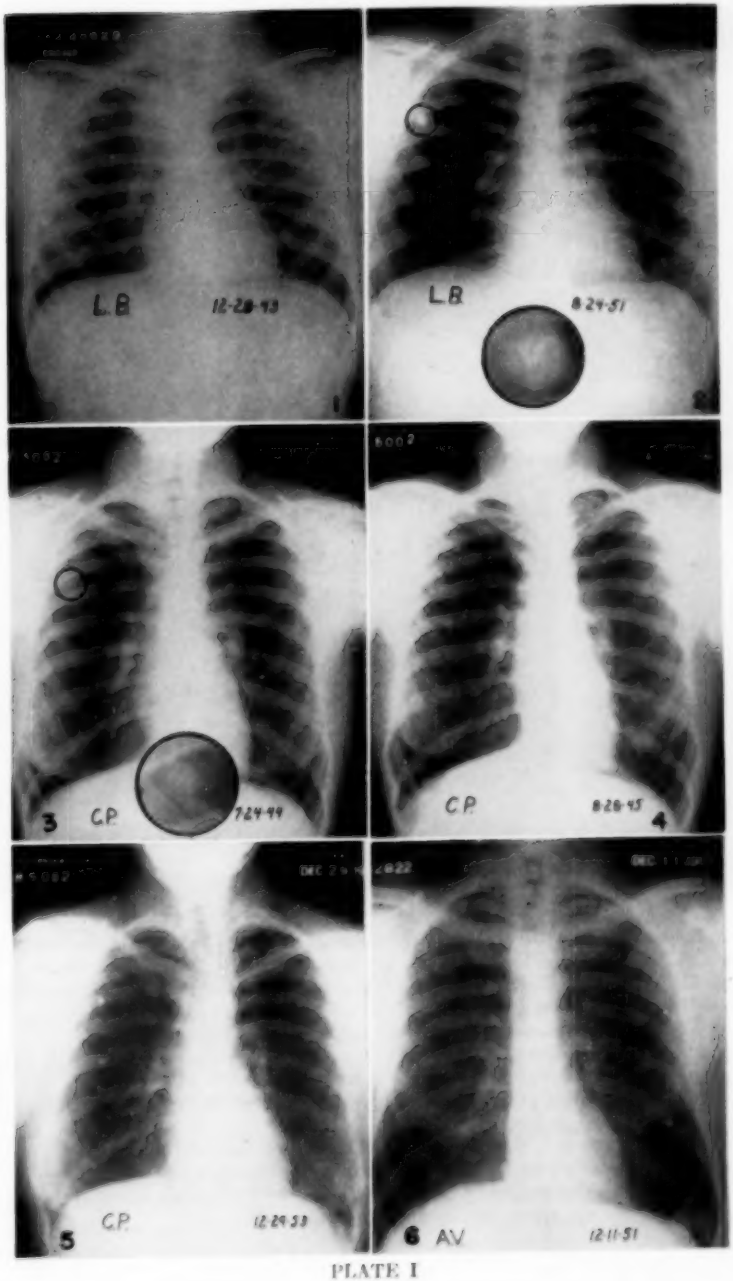
FIG. 2. Roentgenogram of the same patient eight years later. Note lung and hilar calcification which resembles primary tuberculosis.

FIG. 3. Chest film of a 25-year-old housewife (C. P.) in whom a chronic pulmonary disease, characterized by exacerbations and remissions of symptoms, was observed from its onset in 1937. Symptoms included cough, expectoration, fatigue, dyspnea, weight loss, and hemoptysis. The histoplasmin test was positive; the tuberculin test, negative. Complement-fixation reaction for histoplasmosis was positive, with a changing titer which paralleled the clinical course of the disease.

FIG. 4. Same patient one year later. Note extension of the disease in the right mid-lung.

FIG. 5. Same case sixteen years after onset. It is of interest that in 1947 organisms having the morphology of *Histoplasma capsulatum* were demonstrated in two specimens of sputum.

FIG. 6. Chest film of a 27-year-old painter (A. V.) who became ill after exposure to dust near the habitat of domestic fowl. Symptoms included pain in the left shoulder and neck, chills, and fever. The histoplasmin test was positive; the tuberculin test, negative. Complement-fixation reaction for histoplasmosis was positive, with a falling titer. Note roentgenographic evidence of pulmonary cavity in the left lower lung.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1
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A.V.
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PLATE I

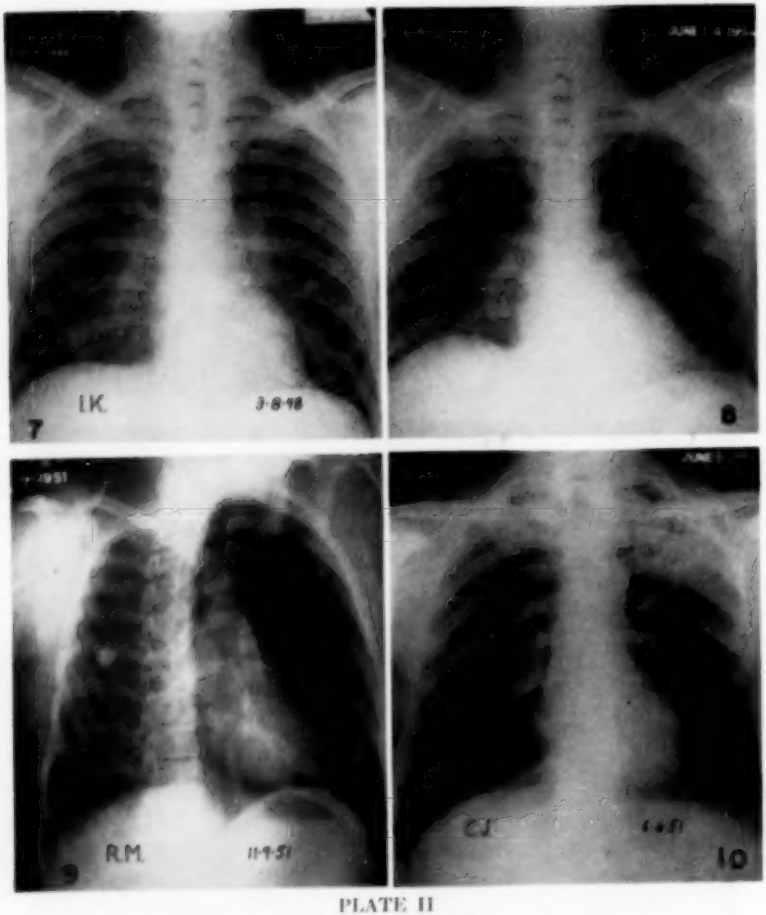

PLATE II

FIG. 7. Chest film of a 32-year-old dairy farmer (I. K.) who became ill (after cleaning and filling a silo) with chills, fever, headache, lassitude, and weight loss. The histoplasmin test was positive; the tuberculin test, negative. Complement-fixation reaction for histoplasmosis was positive, with a falling titer.

FIG. 8. Same patient six years later. Note beginning of calcification.

FIG. 9. Oblique chest film of a 37-year-old woman (R. M.) in whom asymptomatic nodular pulmonary disease was discovered on the right. Histoplasmin and tuberculin skin tests were positive. Organisms having the morphology of *Histoplasma capsulatum* were seen in the lesions after surgical removal.

FIG. 10. Chest film of a 56-year-old man with bilateral cavitary lung disease. This case was previously reported by Monroe and Kurung (9). Histoplasmin and tuberculin skin tests were positive. Complement fixation reaction for histoplasmosis was negative. *Histoplasma capsulatum* was repeatedly cultured from the sputum.

ON THE NATURE OF GENETIC RESISTANCE TO TUBERCULOSIS IN THE LIGHT OF THE HOST-PARASITE RELATIONSHIPS IN NATIVELY RESISTANT AND SUSCEPTIBLE RABBITS[1,2]

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(Received for publication May 12, 1955)

INTRODUCTION

In previous studies on quantitative inhalation of isolated tubercle bacilli (1, 2), evidence was presented which indicated that each bovine tubercle bacillus particle of maximum virulence for the rabbit which reached an alveolar space produced a grossly visible primary pulmonary tubercle irrespective of the native resistance of the host. The chief difference between rabbit races was the chronicity and localization of the ensuing disease. In the susceptible animal the tuberculosis progressed rapidly in the lung, became generalized, and led shortly to a fatal issue. In the resistant rabbit the primary foci at the portal of entry became encapsulated; few, if any, secondary foci of lymphogenous or hematogenous origin developed; and the animal died of localized ulcerative phthisis of long duration.

By contrast, if these resistant and susceptible rabbits inhaled human-type tubercle bacilli under the same conditions, only a small fraction of the microorganisms which penetrated the terminal passages of the lung generated primary pulmonary foci. Furthermore, the greater the native resistance of the rabbit to the bovine-type tubercle bacilli, the fewer were the primary pulmonary tubercles produced on inhalation of a given number of human-type tubercle bacilli. Indeed, so regular was this inverse relationship that the number of these primary foci was constant for each race three to five weeks after inhalation. Thus, the resistant race *III* required approximately 1,000 microorganisms, estimated as inhaled, to yield one pulmonary tubercle; whereas, for the susceptible races *C* and *FC*, 70 to 80 tubercle bacilli sufficed to produce such a focus. It is evident, therefore, that, after the inhalation of certain numbers of human-type tubercle bacilli, the susceptible animal may show an extensive pulmonary disease; whereas, the lungs of the resistant animal may be free from tuberculosis. This was found to be true (2).

It was further demonstrated that BCG vaccination, which enhances the *in vivo* capacity of the macrophages to inhibit the intracellular multiplication of the bacilli, reduces the number of primary pulmonary tubercles which result from the inhalation of a given number of human-type bacilli (3). Conversely, the administration of cortisone, which lowers the resistance of the phagocytes against

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[2] This study was aided by grants from the Commonwealth Fund; the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; and the Public Health Service of the Department of Health, Education, and Welfare.

the accumulation of the microorganism in their cytoplasm, increases the number of these primary foci (4). It is clear, therefore, that this response of the host is a delicate, quantitative measure of the resistance of a given race to tuberculosis.

It is the purpose of this communication to further the elucidation of this phenomenon. An attempt was first made to determine whether the virulence of the individual human-type bacillus which penetrates the lung is a significant factor in the genesis of these primary tubercles. The second and major endeavor of the present investigation was to extract, from a detailed study of the fate of these bacilli and the corresponding host responses in these races, the nature of some of the constitutional factors which control resistance and susceptibility to the disease. It was found that the difference in the host-parasite relationship between a susceptible and a resistant rabbit is of the same nature as the difference between two animals of the same native resistance, one of which is under the intense influence of hydrocortisone and the other is not.

Observations

The genesis of primary pulmonary foci on quantitative inhalation of human-type tubercle bacilli as an index of native resistance

Before presenting the results of these studies, it may be of significance to record the accumulated data on the relative native resistance of some of the rabbit races as determined by the number of human-type bacilli (calculated as inhaled) required to generate a single primary pulmonary focus, on the one hand, and their survival time after the intracutaneous inoculation of a standard dose of highly virulent bovine tubercle bacilli, on the other.

It is clear from table 1 that on the basis of both criteria the order of increasing resistance of these races is as follows: *C*, *FC*, *A*, *AD*, and *III*. However, on both bases, *C* and *FC* do not differ statistically and are the most susceptible. The races *A* and *AD* are of intermediate resistance and do not differ from each other significantly. Race *III* is clearly the most resistant race of all. Furthermore, on

TABLE 1

The Relative Resistance of Inbred Rabbit Races as Determined by Their Survival After Intracutaneous Inoculation of Virulent Bovine-Type Tubercle Bacilli (Ravenel) and Their Response to Quantitative Inhalation of Human-Type Bacilli (H37Rv)

Race	Response to Human Type					Response to Bovine Type	
	Number of Rabbits Used	Range of Bacilli Inhaled	Range of Tubercles Generated	Average Number of Bacilli Required to Form One Tubercle	Average Size of Tubercles and Number of Rabbits in Which They Were Measured	Number of Rabbits Used	Survival in Days After 0.1 Mg. of Ravenel
C	30	1,081–45,359	14–1,452	70 ± 14	4.0 ± 0.27 (11)	8	128 ± 16
FC	45	709–53,821	8–1,442	79 ± 9	3.9 ± 0.15 (33)	8	132 ± 16
A	11	14,868–21,146	51–233	230 ± 24	3.8 ± 0.12 (11)	10	198 ± 15
AD	12	25,027–34,697	25–373	362 ± 103	3.2 ± 0.06 (12)	10	197 ± 16
III	71	570–39,700	0–148	1,065 ± 138	3.1 ± 0.11 (52)	8	422 ± 59

the basis of its response to quantitatively inhaled human-type bacilli, it may be said that race *III* is fourteen to thirteen times as resistant as races *C* and *FC* and four to three times as resistant as races *A* and *AD*, respectively. On the ground of its reaction to a standard intracutaneous dose of virulent bovine tubercle bacilli, race *III* is only about three times as resistant as races *C* and *FC* and only about twice as resistant as races *A* and *AD*, respectively. It is evident, therefore, that the response to quantitatively inhaled human-type tubercle bacilli is a much more precise index of the rabbit's resistance to the disease than is a standard inoculation with bovine tubercle bacilli.

The response of these rabbit races to inhaled human-type tubercle bacilli is indicative, not only of the resistance of each race to the *inception* of the disease, but also, to some extent, of its rate of progression in that race. This is suggested by the size of the ensuing primary tubercles. The greater the resistance, the smaller are the resulting tubercles. Therefore, it becomes of great importance to elucidate the pathogenesis of the formation of these primary pulmonary foci in natively resistant and susceptible animals.

THE INDIVIDUAL VIRULENCE OF THE INHALED BACILLI AS A FACTOR IN THE GENESIS OF PRIMARY PULMONARY FOCI

The observations that each bovine tubercle bacillus particle of maximum virulence for the rabbit which penetrates an alveolar space generates a primary pulmonary focus, irrespective of the resistance of the rabbit (2), has been confirmed and extended to rabbit races of all degrees of native resistance. This is demonstrated in table 2. Approximately three bacilli calculated as inhaled produce one tubercle in each race. Since only one of three inhaled particles of the size of tubercle bacilli reach the terminal alveoli (8), it is evident that each microorganism which penetrates the alveolar passages produces a primary pulmonary focus.

Cortisone, which markedly increases the number of primary foci produced by the inhalation of human-type tubercle bacilli, does not increase the number of foci if the rabbit is exposed to bovine bacilli of high virulence (5). No more than one focus can result from the multiplication of a single bacillus, no matter how low the resistance of the phagocyte that harbors it. It was surmised, therefore, that the virulence of each individual bovine bacillus of this culture was of

TABLE 2

THE NUMBER OF ISOLATED BOVINE-TYPE TUBERCLE BACILLI OF MAXIMUM VIRULENCE, ESTIMATED AS INHALED, REQUIRED TO PRODUCE A SINGLE VISIBLE PRIMARY PULMONARY FOCUS IN RABBITS OF DIFFERENT NATIVE RESISTANCE TO TUBERCULOSIS

Race	Resistance	Number of Rabbits Exposed	Number of Isolated Bacilli Estimated as Inhaled	Average Number of Bacilli Yielding One Tubercle
CaC	Susceptible	6	40–462	2.8 ± 0.2
AD	Intermediate	22	90–688	3.4 ± 0.3
III	Resistant	6	216–306	2.9 ± 0.3

such magnitude that, irrespective of the resistance of the individual alveolar phagocyte which had ingested the microorganism, sufficient multiplication of the bacillus took place in each instance to produce a grossly visible tubercle.

Since the human-type tubercle bacillus is of low virulence for the rabbit as a species, it may be understood why most of the bacilli which penetrate the lung fail to multiply sufficiently to yield visible primary pulmonary foci. It is conceivable that some tubercles are formed from the penetration into the alveoli of some bacilli of greater than average virulence for this animal, irrespective of the possible varying resistance of the ingesting phagocytes. If such unusual virulence is a controlling factor in the genesis of these tubercles, it would follow that the far fewer tubercles formed in the resistant than in the susceptible rabbit are the result of the multiplication of only the most highly virulent organisms inhaled by the former. It might be expected, therefore, that the average virulence of human-type tubercle bacilli recovered from a susceptible rabbit would tend to be less than that of the bacilli obtained from a resistant animal.

To answer this question, cultures of human-type tubercle bacilli were obtained from the lungs of the susceptible race *C*, on the one hand, and from the resistant race *III*, on the other, one week after inhalation. This period was chosen because, as will be seen later, this is the time of greatest contrast in the behavior of the microorganism in these races. At this interval the population of the bacilli in the lung of the susceptible rabbits had increased seven fold over the number that penetrated the pulmonary parenchyma. In the resistant rabbit the population had declined to one-third of the number originally seeded in the lung. Eleven to 12 members of the resistant race *III* were then exposed to the quantitative inhalation of tubercle bacilli obtained from these two races, respectively. Five weeks after inhalation the primary tubercles generated in each group were enumerated as previously described.

The results are presented in table 3. It is evident that, irrespective of the origin of the bacilli, whether obtained from a natively resistant or a natively susceptible animal, their tuberculogenic capacity was essentially the same.

Approximately 1,000 tubercle bacilli cultured from the lung of a susceptible *C* rabbit were required to generate one tubercle in the resistant race *III*. Approximately 800 microorganisms recovered from the resistant race *III* rabbit were necessary to produce one visible primary focus in the closely genetically related rabbits of the same race *III*. The difference is obviously not statistically significant; nor was there any difference in the progression of these tubercles in this race whether the infecting bacilli stemmed from a resistant or a susceptible host.

While these data do not allow a definitive answer to the question posed, they strongly suggest that the virulence of the individual bacillus obtained from a natively resistant and a natively susceptible animal, at a time when its behavior is most contrasting in the respective hosts, is not of sufficient magnitude to materially influence its tuberculogenic capacity for the resistant race *III* rabbits. Hence, the large number of tubercles which result from the inhalation of a given number of human-type bacilli in the susceptible rabbit and the small number which are generated in the resistant rabbit cannot be ascribed to differences in virulence of the organisms concerned in the genesis of these foci.

TABLE 3

THE RELATIVE TUBERCULOGENIC CAPACITY OF H37Rv RECOVERED FROM A NATIVELY RESISTANT RABBIT (IIIR 4=25) AND A NATIVELY SUSCEPTIBLE RABBIT (C 14=4) SEVEN DAYS AFTER QUANTITATIVE INHALATION

Rabbit Number	Parents	Number of Bacilli Inhaled	Number of Tubercles Formed	Number of Inhaled Bacilli Required to Produce One Tubercle	Average Diameter
	Rabbits Infected with Strain "C 14=4"				
III 5=27	III 4=36 × III 3=10	6,006	27	222	3.4
III 5=66	III 4=60 × III 4=34	6,468	5	1,293	3.9
III 5=69	III 4=60 × III 4=34	7,014	9	779	3.3
III 5=76	III 4=46 × III 4=34	7,392	6	1,232	2.9
III 5=81	III 4=42 × III 4=34	7,392	2	3,696	1.5
III 5=74	III 4=42 × III 4=34	5,273	13	406	2.7
III 6=2	III 5=24 × III 3=10	5,397	18	300	3.9
III 6=3	III 5=26 × III 3=10	5,366	20	268	3.8
III 6=11	III 5=30 × III 5=23	5,366	9	596	2.3
III 6=14	III 5=26 × III 5=23	5,086	4	1,272	2.3
III 6=21	III 5=26 × III 5=23	4,930	5	986	2.3
Average				1,004 ± 296*	2.9 ± 0.24†
	Rabbits Infected with Strain "IIIR 4=25"				
III 5=43	III 4=60 × III 3=10	1,915	7	274	3.8
III 5=64	III 4=60 × III 4=34	1,966	3	655	2.7
III 5=68	III 4=60 × III 4=34	2,016	3	672	2.3
III 5=72	III 4=60 × III 4=34	1,953	1	1,953	3.5
III 5=79	III 4=44 × III 4=34	2,104	4	526	2.5
III 6=17	III 5=43 × III 5=25	2,117	1	2,117	3.0
III 5=75	III 4=42 × III 4=43	2,502	7	357	3.6
III 6=9	III 5=27 × III 4=43	2,182	6	364	2.3
III 6=4	III 5=26 × III 3=10	1,932	8	242	4.6
III 6=12	III 5=30 × III 5=23	2,405	4	601	3.4
III 6=15	III 5=26 × III 5=23	2,266	2	1,133	3.0
III 6=23	III 5=30 × III 5=23	2,168	8	271	2.9
Average				764 ± 186*	3.1 ± 0.19†

* $P = 0.246$.
† $P = 0.246$.

THE HOST-PARASITE RELATIONSHIPS IN NATIVELY RESISTANT AND SUSCEPTIBLE RABBITS

The Fate of the Bacilli in the Lungs

As the virulence of the individual bacillus inhaled does not seem to be of importance in the genesis of these primary tubercles, one naturally turns to the

host-parasite relationship in an effort to understand the differing behavior of these bacilli in the natively resistant and susceptible animals.

It was shown in a previous study (2) that several weeks after the inhalation of a particular number of bacilli of human type, the population of the microorganism in the lung of the resistant animal was markedly suppressed by comparison with that in the lung of the susceptible animal. The question arises whether this suppression represents a more rapid and effective development of acquired resistance by the natively resistant race, as has been repeatedly demonstrated in this laboratory. On the other hand, the suppression might result from an original and immediate greater restriction of growth of the bacilli in the natively resistant than in the susceptible animal. It is also relevant in this connection to consider histologic responses in these races which might account for the differing behavior of the bacilli in the tissues.

To elucidate the host-parasite relationship in these rabbit races, 31 susceptible and 33 resistant animals were exposed in groups of 6 (3 race *III* rabbits and 3 race *C* rabbits) to the quantitative inhalation of isolated H37Rv. The number of bacilli which these rabbits inhaled varied from 3,000 to 45,000 per animal. One and three days, and one, two, three, four, and eight weeks after inhalation, the rabbits were killed and the lungs and tracheobronchial lymph nodes were weighed. One entire lung was ground in a Waring Blendor with 9 ml. of M/15 disodium acid phosphate per gram of tissue and cultured quantitatively on a modified Löwenstein medium (6), both directly and after treatment with an equal volume of 6 per cent sulfuric acid. The other lung was distended to its full capacity by an intratracheal injection of 10 per cent neutral formalin and stained with hematoxylin-eosin and the Ziehl-Neelsen procedure for tubercle bacilli. The tracheobronchial lymph nodes and spleen were quantitatively cultured directly, the latter organ from the second week after inhalation. At least 4 resistant and 4 susceptible rabbits were studied at each interval. For what was found to be the critical one-week period, 7 animals were employed.

In tables 4 and 5 are presented the detailed numbers of bacilli obtained from both lungs, on direct culture and after treatment, in the susceptible and the resistant races, respectively, at different intervals following the inhalation infection. In the third column of these tables it may be seen that each rabbit in each of these exposures inhaled greatly varying numbers of bacilli. Nevertheless, the average number inhaled by the 4 to 7 animals of each group, exposed for the determination of their response at different intervals following infection, was fairly uniform and varied only from 12,000 to 21,000 microorganisms. This is of importance in the delineation of the dynamic host-parasite relationship at different intervals in these races considered as groups. If this relative constancy had not been achieved, the interpretation of the sequence of events would have been more difficult.

It will be noted that, while treatment with sulfuric acid constantly reduced the number of bacilli cultured from a unit weight of lung, this reduction was relatively consistent. Consequently, the rate of accumulation of bacilli in the lungs as judged by the number recovered on direct culture was approximately two to three fold that obtained after treatment. This relation supports the essential reliability of the values obtained on the growth and destruction of the bacilli at different intervals following infection.

TABLE 4

THE RATE OF ACCUMULATION OF HUMAN-TYPE BACILLI (H37Rv) IN THE LUNGS OF RESISTANT AND SUSCEPTIBLE RABBITS AT DIFFERENT INTERVALS FOLLOWING SIMULTANEOUS AIRBORNE INFECTION

Susceptible Race C

Number of Days After Exposure	Rabbit Number	Number of Bacilli Estimated as Inhaled	Number of Viable Bacilli Estimated in Lungs		Ratio Between Number Seeded and Recovered	
			Direct	Treated	Direct	Treated
1 day	C 12=40	8,920	680	532	0.20	0.20
	C 13=9	5,200		498		0.30
	C 13=35	3,140	465	78	0.40	0.07
	C 14=1	37,950	2,978	1,645	0.20	0.10
	Average	13,803	1,374	688	0.27	0.17
3 days	C 13=6	6,440		238		0.10
	C 12=10	6,110	1,479	609	0.70	0.30
	C 13=28	5,200		70		0.04
	C 14=3	45,200		1,230		0.10
	Average	15,738	1,479	537	0.70	0.14
1 week	C 13=1	9,360	8,500	4,250	2.70	1.4
	C 13=11	6,275	17,160	11,310	8.20	5.4
	C 13=33	2,930	1,760	1,176	1.80	1.2
	C 14=4	40,300		12,032		0.9
	C 14=19	31,250	150,600	88,920	15.30	8.5
	C 14=23	28,600	77,000	26,180	8.10	2.7
	C 14=24	27,400	67,200	20,640	7.40	2.3
	Average	20,874	55,203	23,501	7.30	3.2
2 weeks	C 13=9	5,860	3,450,000	1,495,000	1,706	765
	C 12=19	6,275	5,762,000	2,141,400	2,754	1,024
	C 13=27	5,460	1,034,000	131,600	568	72
	C 14=5	35,400		3,876,000		329
	Average	13,249	3,415,333	1,911,000	1,696	548
3 weeks	C 13=3	8,920	5,830,000	4,388,400	1,964	1,476
	C 13=14	6,100	77,760,000	40,320,000	38,249	19,833
	C 13=32	3,240	41,070,000	10,989,000	38,028	10,175
	C 14=16	41,750	149,600,000	45,560,000	10,749	3,275
	Average	15,003	68,565,000	25,314,350	22,248	8,690
4 weeks	C 13=21	5,710	27,018,000	3,579,600	14,198	1,881
	C 12=38	5,700	87,360,000	13,377,000	45,979	7,041
	C 12=35	6,350	51,920,000	15,812,000	24,525	7,469
	C 14=22	33,800	65,600,000	25,092,000	5,840	2,235
	Average	12,890	57,974,500	14,465,150	22,636	4,657
8 weeks	C 13=2	9,650	16,200,000	5,400,000	5,036	1,678
	C 13=38	9,900	42,750,000	14,535,000	12,955	4,405
	C 13=39	9,140	24,570,000	11,466,000	8,064	3,763
	C 13=37	20,150	18,340,000	9,379,600	2,741	1,396
	Average	12,210	25,465,000	10,195,150	7,199	2,811

TABLE 5

THE RATE OF ACCUMULATION OF HUMAN-TYPE BACILLI (H37Rv) IN THE LUNGS OF RESISTANT AND SUSCEPTIBLE RABBITS AT DIFFERENT INTERVALS FOLLOWING SIMULTANEOUS AIRBORNE INFECTION

Resistant Race III

Number of Days After Exposure	Rabbit Number	Number of Bacilli Estimated as Inhaled	Number of Viable Bacilli Estimated in Lungs		Ratio Between Number Seeded and Recovered	
			Direct	Treated	Direct	Treated
1 day	III 5=34	12,390		615		0.10
	III 5=50	7,470	2,400	1,000	1.00	0.40
	IIIR4=21	3,400	735	52	0.60	0.05
	IIIR4=22	42,100	4,601	1,260	0.30	0.10
	Average	16,340	2,579	732	0.60	0.16
3 days	III 5=51	7,140		612		0.30
	III 5=3	6,570	776	33	0.40	0.01
	IIIR4=15	5,350	680	43	0.40	0.02
	IIIR4=24	45,500		564		0.04
	Average	16,140	728	313	0.40	0.09
1 week	III 5=37	6,630	792	158	0.20	0.06
	III 5=61	7,600	1,631	273	0.60	0.10
	IIIR4=18	3,320	150	75	0.10	0.07
	IIIR4=25	43,100		583		0.04
	III 6=30	28,600	6,020	2,580	0.60	0.30
	III 6=34	30,100	609	148	0.06	0.01
	III 6=37	30,300	736	156	0.07	0.01
	Average	21,521	1,656	568	0.27	0.08
2 weeks	III 5=52	6,630	70,400	74,800	32	34
	III 5=41	6,939	195,500	74,375	85	32
	IIIR4=14	6,900	29,200	18,240	13	8
	IIIR4=26	35,700	1,062,500	500,000	89	42
	Average	14,042	339,400	166,854	55	29
3 weeks	III 5=44	12,120	2,700,000	837,000	661	207
	III 5=62	7,300	5,250,000	2,407,000	2,158	980
	IIIR4=16	3,070	175,500	38,610	171	37
	IIIR5=2	39,700	6,405,000	1,708,000	484	121
	Average	15,548	3,632,625	1,247,653	869	339
4 weeks	III 5=54	6,400	2,900,000	1,040,000	1,359	488
	III 5=58	7,320	3,060,000	765,000	1,254	314
	IIIR4=12	6,500	2,185,000	1,092,500	1,008	504
	IIIR5=3	41,750	6,832,000	7,503,000	491	540
	Average	15,493	3,749,250	2,600,125	1,028	462
8 weeks	IIIR5=4	10,100	62,040	39,204	18	12
	IIIR5=6	9,500	361,800	134,000	114	42
	IIIR5=12	11,300	673,200	448,800	179	119
	III 6=24*	26,350	7,791,000	4,346,500	888	495
	III 6=25	25,400	192,030	244,200	23	29
	III 6=32*	19,780	4,200,000	2,593,500	637	393
	Average	17,072	2,213,345	1,301,034	310	182

* Cavity

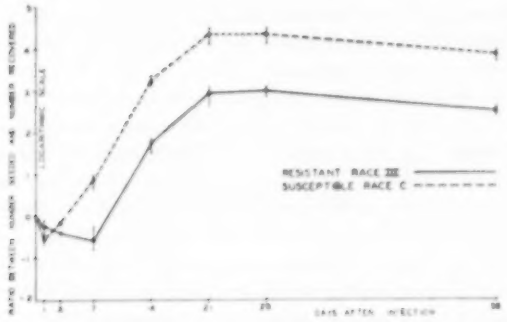

FIG. 1. Population changes of human-type bacilli (H37Rv) in the lungs of natively resistant and susceptible rabbits at different intervals after quantitative airborne infection (direct culture).

For greater ease of examination of the population changes in the lungs of the resistant and susceptible rabbits, these data are plotted in figures 1 and 2. The number of bacilli inhaled by each rabbit can be estimated (7) and only one-third of the inhaled bacilli penetrated the alveoli (8). Hence, the two graphs present the average ratio which existed between one-third of the bacilli estimated as originally inhaled by each group and the average number of bacilli cultured from both lungs of the same group at different intervals after infection. The ratios for each rabbit are presented in tables 4 and 5. These changes as obtained on direct culture of the lung are plotted in figure 1. In figure 2 the data obtained after sulfuric acid treatment are depicted. Both graphs include the standard deviation of each mean for each interval.

Inspection of these curves recalls the close similarity which exists between the behavior of the bacilli in the lungs of both types of animals and the behavior of bacteria in an artificial medium. There is a lag phase in bacillary accumulation, a phase of logarithmic multiplication, and one of decline. It is also immediately

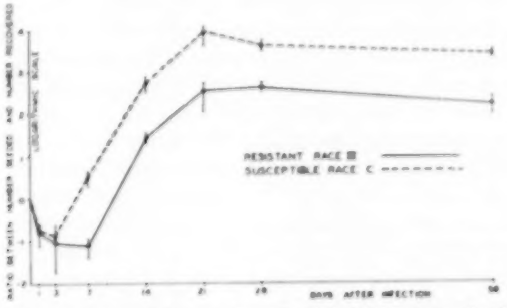

FIG. 2. Population changes of human-type bacilli (H37Rv) in the lungs of natively resistant and susceptible rabbits at different intervals after quantitative airborne infection (treated culture).

evident that the lung of the resistant animal is a poorer medium for the growth of the bacilli than that of the susceptible animal throughout the course of the infection and from its very beginning.

Unfortunately, it is not always possible to obtain direct cultures of tubercle bacilli from the lungs in the first few days following infection, as other bacteria are frequently present in the bronchi. When the tubercle bacilli have multiplied and dilutions of the lung brei are cultured, the contaminating bacteria are eliminated by dilution and the tubercle bacilli are invariably recovered in pure culture. It was shown that treatment of the lung with sulfuric acid reduced the number of bacilli cultured to approximately a half or a third of that obtained on direct culture. The absolute bacillary population is not the major concern in these experiments, however; it is the relative changes in this population which are of interest. Hence, it is admissible to estimate the behavior of the population in the first few days of the infection on the basis of the colony counts recovered after treatment.

A close scrutiny of the tables and charts will reveal that only about one-half to one-third of the bacilli estimated as penetrating the lung were recovered one day after inhalation on direct culture, and about one-sixth of these microorganisms were obtained from the treated lung at this time. Two days later the population of the lung in both the resistant and susceptible races had not increased. In the resistant race *III*, their numbers had perhaps declined still further.

The most impressive difference in the growth of the bacilli in the lungs of these two races occurred one week after inhalation. At this time, in 7 susceptible rabbits the population had increased seven fold over the original number seeded in their lung parenchyma. In the 7 simultaneously exposed resistant animals, the total number of bacilli still represented only one-third of those originally trapped in the lung. Thus, at this early period, the inhibition to multiplication was twenty-one times greater in the resistant than in the susceptible animal.

The lag phase in the susceptible animal, therefore, lasted only three days. In the resistant animal it lasted at least seven days. After this varying lag phase, logarithmic growth took place in both races; but again, while the population of the bacilli in the lung of the susceptible animal had increased approximately seventeen hundred fold two weeks after infection, in the resistant animal the census had increased only fifty-five fold. Again, the obstruction to multiplication was approximately thirty times greater in the resistant than in the susceptible rabbit.

Three weeks after infection the population of the bacilli in the lung was still rising in both races. However, in the susceptible animal it had increased to more than twenty-two thousand fold over the original number seeded. In the resistant animal this increase was only about eight hundred fold, or approximately twenty-five times less. At the fourth week, bacillary accumulation ceased in the lungs of both races. Again, however, the population in the lung of the susceptible animal was still approximately twenty fold greater than that of the resistant rabbit as compared with the original number seeded in each. Eight weeks after infection the bacilli in the lungs of both animals had declined. Just as at previous in-

tervals, the residual microorganisms in the susceptible animals were still twenty-three fold greater than those in the resistant animals, by the above index. This occurred despite the development of softening in the lesions of the resistant animals, a process which tends to greatly increase the bacillary population. This subject will be described below.

It is noteworthy that essentially similar trends are in evidence whether one studies the data obtained from direct culture of the lung or after treatment. This may be seen from a comparison of figures 1 and 2.

Thus, the essential difference in the behavior of the bacilli in these two races of rabbits is not the fact that the resistant animal completely prevents the growth of the bacilli and that the susceptible animal permits them to grow unhindered, but rather that the inhibition is much more pronounced in the former throughout the course of the infection and from its very beginning. This bacillary restriction is approximately twenty to thirty times greater in the natively resistant race during both the growth phase and the period of decline. It is this greater inhibitory factor in the resistant animal which, by itself, accounts for the prolongation of the lag period in this race and the earlier onset of logarithmic growth in the susceptible strain.

Since logarithmic increase in the bacillary population took place in the resistant animal in the interval between seven and fourteen days, no effective acquired resistance could be in force at that time. Therefore, the preceding longer lag phase which differentiates the response of the resistant from the susceptible animal cannot be attributed to a more rapidly acquired resistance by the former. This differing bacillary growth must be an expression of nonspecific constitutional factors which initially inhibit the multiplication of the bacilli in the resistant animal to a much greater degree than in the susceptible animal.

An attempt was not made to define the significance of this behavior difference of the bacilli in the resistant and susceptible animals in relation to the number of tubercles generated in these two races by a given number of human-type tubercle bacilli.

In table 6 may be seen the number of grossly visible primary foci found in the lungs of these rabbit series three to four weeks after inhalation. The 8 susceptible *C* rabbits required an average of 61 tubercle bacilli estimated as inhaled to generate a tubercle. The 8 resistant race *III* rabbits required an average of 816 bacilli (Plate I, figures 1 and 2).

It was stated above that there is convincing evidence that each bovine tubercle bacillus of maximum virulence which penetrates the terminal alveolar passages of the lungs of resistant or susceptible animals multiplies sufficiently to yield a primary pulmonary focus. With human-type tubercle bacilli, only a small fraction of these multiply to that extent, and this varies with the resistance of the host. The question arises whether each microorganism which fails to multiply necessarily dies. Reference to table 4 shows that, if all of the bacilli which remained alive in the susceptible *C* rabbits three days after infection (the end of the lag phase) had multiplied sufficiently in different sites of localization to yield a focus, an average of 1,500 foci would have developed. In actuality, only 241

TABLE 6

THE NUMBER OF TUBERCLES GENERATED IN RESISTANT RACE III AND SUSCEPTIBLE RACE C THREE TO FOUR WEEKS FOLLOWING SIMULTANEOUS QUANTITATIVE INHALATION OF H37Rv

Rabbit Number	Weeks After Inhalation	Number of Bacilli Estimated as Inhaled	Number of Tubercles Generated	Number of Inhaled Bacilli Required to Produce One Tubercle
		Resistant Race III		
III 5=44	3	12,120	14	866
III 5=62	3	7,300	28	261
IIIR4=16	3	3,070	2	1,535
IIIR5=2	3	39,700	23	1,726
III 5=54	4	6,400	26	246
III 5=58	4	7,320	41	179
IIIR4=12	4	6,500	6	1,083
IIIR5=3	4	41,750	66	633
Average		17,270	26	816 ± 212
		Susceptible Race C		
C 13=3	3	8,920	113	79
C 13=14	3	6,100	196	31
C 13=32	3	3,240	44	73
C 14=16	3	41,750	734	57
C 13=21	4	5,710	121	47
C 12=38	4	5,700	185	31
C 12=35	4	6,350	67	95
C 14=22	4	33,800	471	72
Average		13,946	241	61 ± 8

such tubercles were found. Therefore, only about one-sixth of these microorganisms thus prospered. The remainder were alive for some time but did not multiply sufficiently. Likewise, reference to table 5 indicates that, if all of the bacilli which remained alive at the end of seven days (the lag period for the resistant animals) had multiplied sufficiently in the different sites to produce a tubercle, approximately more than 1,600 such foci would have been generated. In reality, only 26 tubercles, on the average, were found in these animals. Therefore, only about one-sixtieth of the surviving bacilli succeeded in multiplying to that extent; the remainder failed to reproduce to the required degree.

These data suggest, therefore, that human-type tubercle bacilli ingested by alveolar phagocytes in the rabbit do not merely either multiply or die but persist for a considerable time without multiplying. Furthermore, the data also suggest that the obstruction to multiplication is greater in many more bacilli-bearing phagocytes before the formation of a visible tubercle in the resistant than in the susceptible animal. This is the simplest explanation of the observation that many more tubercles are generated in a susceptible host which inhales a given number of human-type bacilli than are generated in a resistant host.

The question now arises, is the multiplication of the bacilli in the sites where sufficient growth does take place to produce visible tubercles in the resistant and susceptible animals of the same or different degree. Reference to the graphs and their analysis made above indicates that, whereas the obstruction to multiplication of the bacilli was approximately twenty to thirty fold greater in the resistant than in the susceptible animal, the reduction in the formation of tubercle in the resistant race was only about thirteen fold. It must be concluded, therefore, that, even in the sites at which the bacilli succeeded in multiplying sufficiently to yield visible tubercles, there was more effective inhibition of multiplication in the resistant than in the susceptible animals. These over-all quantitative data are amply confirmed by the histologic study of these lesions as discussed below.

The Fate of the Bacilli in the Tracheobronchial Lymph Nodes and Spleen

Lymphogenous and hematogenous dissemination of the tubercle bacilli from the portal of entry in the lung is very much greater in susceptible than in resistant rabbits after quantitative inhalation of virulent bovine bacilli. It is of interest, therefore, to determine whether this difference in dissemination is due to the fixation of the bacilli at the portal of entry in the resistant animal and their dispersion from this site in the susceptible animal. Alternately, the difference in dissemination might result from failure of the bacilli to survive long in the metastatic foci of the resistant animal and their continued proliferation in the metastatic foci of the susceptible animal.

In figure 3 may be seen the average number of bacilli cultured from 100 mg. of tracheobronchial lymph nodes draining the lungs of the 4 to 7 rabbits at each of the seven intervals (one to fifty-six days) following infection in the resistant and susceptible races. It will be seen that no bacilli were recovered from these nodes one and three days after inhalation in either race. One week after infection small numbers of bacilli reached the nodes of both races of rabbits. At the end of the second week large numbers of microorganisms (and in equal quantities) were recovered per unit weight of node in each race.

The population of the bacilli in the nodes of the susceptible animals three weeks after infection continued to rise. In the resistant animals this accumulation rate was considerably less than in the susceptible race. Four weeks after

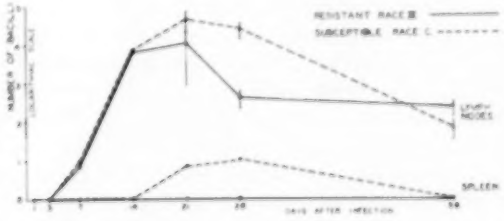

FIG. 3. The number of bacilli recovered by direct culture from 100 mg. of tracheobronchial lymph nodes and 100 mg. of spleen in natively resistant and susceptible rabbits at different intervals after simultaneous quantitative airborne infection.

infection, the population of the microorganisms in the nodes of the susceptible animal was reduced to approximately one-half of that present in these nodes the week before. At the same interval in the resistant race the bacilli were reduced to one twenty-seventh of their previous number. Therefore, the reduction was approximately fourteen times greater in the resistant race.

At the end of two months, the bacilli in the tracheobronchial lymph nodes of the susceptible animals had been reduced to very few survivors, which constituted about one four-hundredth of the number remaining there four weeks after infection. In the resistant animals, however, this corresponding reduction was less than two fold.

This entirely unexpected finding may be understood if it be assumed that the bacilli cultured from these nodes at this time had recently arrived from the lungs which they drained. As will be seen later, two months after infection, the few pulmonary tuberculous foci present in the lungs of the resistant animals were undergoing softening (Plate I, figure 4). This was accompanied by an intense extracellular multiplication of the microorganism in the liquefied caseous tissue (Plate I, figure 5). These lesions were in intimate relation to the circulation. In the susceptible animals, on the other hand, little, if any, liquefaction of the much more numerous avascular caseous lesions took place (Plate I, figure 3).

It is clear, therefore, that fixation of the bacilli at the portal of entry in the resistant animal is not the underlying mechanism which prevents the dissemination of the disease in this race. The bacilli invade the draining nodes of the resistant animal at least as rapidly as they do the nodes of the susceptible rabbit. The evidence at hand suggests that it is rather the more rapid destruction of the bacilli focalized in the resistant animal, as seen in the four-week interval noted above, which accounts for the failure of metastatic foci in this race to take root.

It is of interest to analyze the bacillary population of the lymph nodes of these two races in relation to the number of bacilli present in the corresponding lungs of these strains. When this is done, it may be seen that the rate of dispersion of the bacilli from the portal of entry is much greater in the resistant than in the susceptible animal. This relationship is presented in table 7.

It will be noted that one week after infection approximately 3 per cent of the bacilli cultured from 1 gm. of lung of the resistant animal are obtained from 100 mg. of its lymph nodes. Only 0.2 per cent of the pulmonary population of the susceptible animal are cultured from its nodes at this time. Two weeks after inhalation approximately the same number of bacilli were cultured from the nodes of both races. Nevertheless, at this time, 1 gm. of lung of the susceptible animals contained, on the average, approximately ninety-seven times as many bacilli as a corresponding weight of lung of resistant rabbits. The total weights of these lungs were insignificant in this respect. In the resistant rabbit the bacilli in the nodes constituted more than 20 per cent of the pulmonary population. In the susceptible animal they represented only 1 per cent of this corresponding population.

It has already been demonstrated that the tissues of the resistant animal

TABLE 7

THE NUMBER OF BACILLI CULTURED FROM 100 MG. OF SPLEEN AND 100 MG. OF TRACHEOBRONCHIAL LYMPH NODES AND THE RATIO BETWEEN THE NUMBER OF BACILLI IN THE LYMPH NODES AND THOSE IN THE LUNG, PER UNIT WEIGHT OF EACH, IN PER CENT

Interval, weeks	Resistant Race III					Susceptible Race C				
	Rabbit Number	Number of Bacilli in 100 Mg. of Spleen	Number of Bacilli in 1 Gm. of Lung Tissue	Number of Bacilli in 100 Mg. of Lymph Node	Ratio Node/Lung	Rabbit Number	Number of Bacilli in 100 Mg. of Spleen	Number of Bacilli in 1 Gm. of Lung Tissue	Number of Bacilli in 100 Mg. of Lymph Node	Ratio Node/Lung
1	III 5=37		83	0		C 13=1		1,000	0	
	III 5=61		179	0		C 13=11		2,260	0	
	IIIR4=18		16	0		C 13=33		226	0	
	IIIR4=25		207	47		C 14=4		2,343	5	
	III 6=30		700	3		C 14=19		14,000	7	
	III 6=34		70	0		C 14=23		10,000	12	
	III 6=37		80	0		C 14=24		7,000	36	
	Average		191	7	3.1	Average		5,261	9	0.2
2	III 5=52	0	8,000	15,544		C 13=9	0	300,000	7,866	
	III 5=41	0	22,300	8,700		C 12=19	0	672,000	4,900	
	IIIR4=14	0	2,000	100		C 13=27	0	110,000	100	
	IIIR4=26	0	85,000	0		C 14=5	0	1,254,000	14,200	
	Average	0	29,475	6,086	20.6	Average	0	584,000	6,767	1.2
3	III 5=44	0	200,000	550		C 13=3	0	550,000	13,300	
	III 5=62	3	700,000	43,400		C 13=14	7	5,330,000	15,700	
	IIIR4=16	0	15,000	1,100		C 13=32	18	3,700,000	122,000	
	IIIR5=2	0	525,000	1,100		C 14=16	2	10,950,000	40,000	
	Average	1	360,000	11,538	3.2	Average	7	5,132,500	47,750	0.9
4	III 5=54	0	290,000	250		C 13=21	10	2,370,000	12,000	
	III 5=58	3	340,000	450		C 12=38	5	9,600,000	26,700	
	IIIR4=12	0	190,000	1,000		C 12=35	25	4,400,000	58,000	
	IIIR5=3	0	560,000	30		C 14=22	5	4,000,000	6,818	
	Average	1	345,000	433	0.1	Average	11	5,092,500	25,880	0.5
8	IIIR5=4	0	4,700	73		C 13=2	0	1,200,000	56	
	IIIR5=6	0	26,900	503		C 13=38	0	2,500,000	160	
	IIIR5=12	0	66,000	448		C 13=39	1	2,100,000	40	
	III 6=24	0	530,000	30		C 13=37	0	1,400,000	10	
	III 6=25	0	17,300	0						
	III 6=32	2	400,000	228						
	Average	1	240,817	229	0.1	Average	1	1,800,000	67	0.004

inhibit the multiplication of the bacilli much more than do those of the susceptible rabbit. Consequently, it is evident that the far greater proportion of bacilli in the nodes of the resistant than the susceptible animal cannot be attributed to a greater proliferation of the bacilli brought to the draining nodes of the former. This phenomenon can have only one meaning, namely, that the bacilli from the pulmonary foci of the resistant animal are conveyed to the draining lymph nodes in much greater numbers than those from the foci of the susceptible animal. The same general tendency obtains at all intervals. The special conditions prevalent at the four- and eight-week intervals were already discussed above.

Except for an occasional bacillus in a rare individual animal, no microorganisms were cultured from the spleens of the resistant race at any time during the course of the infection (table 7). From the susceptible rabbits some bacilli were obtained from almost all of the spleens cultured in the third and fourth weeks after inhalation. By the eighth week practically no bacilli survived in the spleens of these animals.

It is evident, therefore, that far from a greater fixation of the bacilli at the portal of entry in the resistant than in the susceptible animal, the reverse is actually true. But, because of the greater native and acquired inhibition of bacillary growth by the tissues of the resistant than of the susceptible animal, they soon perish in the metastatic foci of the former and survive longer in the latter.

The Tissue Responses in the Resistant and Susceptible Races

No specific microscopic changes could be identified with certainty in the lungs of the resistant or susceptible animals before the second week after inhalation of H37Rv under the conditions of these experiments. No tubercle bacilli were found in the lungs on histologic examination in any of these rabbits before the second week. Nevertheless, an average of more than 55,000 microorganisms were recovered from the entire lung of the susceptible animals and more than 1,600 were cultured from the lungs of the resistant animals one week after the infection. This discrepancy is probably due to the extreme dispersion of the isolated, relatively small numbers of bacilli in the relatively tremendous area which constitutes the total respiratory alveolar surface.

Occasionally, primitive tuberculous foci were found in the lungs of the susceptible and resistant rabbits two weeks after inhalation. Such a focus in a susceptible animal is illustrated in Plate II, figure 6; and in Plate II, figure 7, a similar lesion in a resistant animal is depicted. In the susceptible animal approximately six adjoining alveolar spaces were filled with large *intact* mononuclear cells with reticulated cytoplasm and vesicular nuclei harboring large numbers of tubercle bacilli. Many of the bacilli were in the form of dense skeins of deeply acid-fast parallel rods. An occasional polymorphonuclear cell or lymphocyte was interspersed between these young epithelioid cells. The alveolar septa were slightly thickened, and an occasional neighboring vessel showed marked cuffing, both due to an accumulation of proliferating mononuclears in which tubercle bacilli could be seen.

In the resistant animal the corresponding lesion occupied two communicating alveolar spaces in which similar uninjured macrophages also harbored tubercle bacilli, but in much smaller numbers than those seen in the susceptible animals. Clumps of rapidly growing bacilli were less in evidence. Numerous polymorphonuclear cells had invaded the alveolar space. Vascular cuffing was noted and the alveolar septa were thickened to a much greater degree than those of the susceptible animal, but no bacilli were seen in these interstitial infiltrating mononuclears.

Three weeks after infection the tubercles in the susceptible rabbit were composed of large *immature* epithelioid cells with intercommunicating processes with reticulated cytoplasm harboring extremely large numbers of bacilli, either isolated or in parallel packets. The young epithelioid cells were surrounded by large zones of rapidly growing mononuclears interspersed with numerous polymorphonuclear cells and pronounced exudation of macrophages into the alveolar spaces. In some instances the centrally situated immature epithelioid cells were undergoing injury and the first stages of caseation with marked infiltration of dead and dying polymorphonuclear cells. The bacilli here were in great numbers, indeed, and could be seen on low power. Some capillaries were beginning to invade the tubercle. Many of the alveolar phagocytes at the periphery of the tubercle still carried intact bacilli and thus spread the disease. Such a focus is illustrated in Plate II, figure 8.

In the resistant rabbits at this time, the foci were much smaller and the bacilli were invariably much fewer and were seen as one or two rods in *mature* rounded epithelioid cells. These had no intercommunicating processes; the cytoplasm was abundant, extremely finely vacuolated, and differentiated into an inner lighter spherical zone and an outer more basophilic periphery. Caseation was less extensive. The perifocal cellular inflammation was predominantly interstitial, polymorphonuclear leukocytes were not abundant, and the capillary ingrowth was pronounced (Plate II, figure 9).

Four weeks after inhalation, in the susceptible rabbits large tuberculous foci were seen which were predominantly pneumonic in character, with numerous intra-alveolar plugs of caseous tissue which still contained much nuclear debris. The interstitial component of the inflammatory process was less extensive. Vascularization was present with stasis of erythrocytes in some of the ingrowing capillaries. Tubercle bacilli in large numbers, and often in clumps, were present in the prominent caseous centers. Bacilli were also present in the cytoplasm of the cells at the transition zone between the living and the dead tissue (Plate III, figures 10 and 12).

In the resistant rabbit, the interstitial process predominated. The caseation was further advanced, with little residual nuclear debris. Bacilli were few and isolated (Plate III, figures 11 and 13). Giant cells and lymphocytes were beginning to accumulate at the the periphery of the tubercle. The capillaries often penetrated to the very center of the caseous tissue and fibroblasts began to appear. In an occasional tubercle the first stages of liquefaction of the caseum or disintegration of the epithelioid cells could be discerned.

Eight weeks after infection the very large and, at times, confluent tubercles

showed extensive caseous centers which were compact and amorphous. Often the original alveolar spaces in which the caseous plugs were situated could be detected. There was little if any trace of liquefaction. Extensive tuberculous granulation tissue was noted. Giant cells were rare and lymphocytic accumulation was not pronounced. The bacilli still swarmed in the large caseous masses. Vascularization was moderate, and stasis of erythrocytes was often noted. Fibrosis was slightly developed (Plate I, figure 3; Plate IV, figures 14 and 15).

In the resistant rabbit the few foci encountered were of two varieties. Some were small, inconspicuous epithelioid cell tubercles without caseation which were rapidly disintegrating by the penetration of the vigorously ingrowing capillaries or by resorption of the cytoplasm of the epithelioid cells, with the consequent development of large spaces between them. These were infiltrated with either large numbers of erythrocytes or polymorphonuclear leukocytes and lymphocytes. Such lesions leave no trace behind them, and the affected lung parenchyma characteristically returns to its original normal structure.

The other type of focus noted in resistant rabbits consisted of large foci. These at times were 6 to 8 mm. in diameter and could be seen in the gross as well walled-off cavities. The caseous foci were undergoing extensive liquefaction. At times this liquefaction was of the sequestrating type and the edge of the cavity was smooth, as if cut out with a knife. On other occasions the edge of the cavity was a ragged septic membrane composed of necrotic tissue, disintegrating epithelioid cells infiltrated with large numbers of dead and dying lymphocytes and polymorphonuclear leukocytes. Large giant cells, both of the Langhans' and the foreign-body type, were seen at the points of transition. At times these cells contained aggregated bacillary masses, especially the latter cells.

In the softened and liquefied necrotic material, the bacilli were often swarming in great numbers outside of the influence of living tissue (Plate I, figure 5). Rupture of the liquefied tissue into bronchi could be discerned. The granulation tissue forming the wall of these cavities was intensely vascularized, with extreme congestion of the vessels. No stasis and fusion of the erythrocytes were noted in these vessels. Dense layers of fibroblasts were deposited here and there in this granulation tissue wall. Some showed the laying down of collagenous fibrillar tissue. Large masses of lymphocytes were accumulated in this granulation tissue. Often lymphatic vessels were crowded with large agglomerations of lymphocytes. It is evident that both the blood and lymph flow to and from these foci was very active (Plate I, figures 4 and 5; Plate IV, figures 16 and 17).

In the lymph nodes draining the lungs of the susceptible rabbits, no bacilli were found two weeks after infection nor were any tuberculous foci encountered. In the resistant rabbits, some of the tracheobronchial lymph nodes and, occasionally, an intrapulmonary node revealed large mononuclear cells with some bacilli. Three weeks after infection few foci of immature epithelioid cells, with few bacilli, were seen in the lymph nodes of both races. In the resistant race, these epithelioid cells were at times more mature. Four weeks after infection a considerable proportion of the lymph node parenchyma of the susceptible rabbits was replaced by epithelioid and giant-cell tubercles with minute specks of

caseation and small numbers of bacilli. In the resistant animals many of the lymph nodes showed either little, if any, tuberculous changes or merely residual tubercles undergoing disintegration. By the eighth week the lymph nodes of both races had returned to normal except for an occasional regressive epithelioid cell with fuchsinophilic cytoplasm. No bacilli were seen. It is noteworthy that the sinuses of the lymph nodes in the resistant animal at this interval contained large numbers of erythrocytes apparently derived from the disintegrating tubercles of the lung which, as stated above, were frequently infiltrated with large numbers of erythrocytes from the ingrowing injured capillaries.

Thus, it is evident that there is a constant and considerable difference in the response of the tissues of these two races to the inhalation of human-type tubercle bacilli. From the beginning, the intracellular accumulation of bacilli is much more pronounced in the intra-alveolar macrophages of the susceptible than of the resistant animal. Except for one interval late in the course of the disease, the bacilli are invariably less numerous not only in the living cells of the resistant rabbit but also in the caseous foci which develop in both races.

The maturation of the epithelioid cells is more rapid in the resistant than in the susceptible animal. The development of the caseous process is, likewise, accelerated in the resistant animal. Pneumonic exudation predominates in the inflammatory process of the susceptible animal. In the resistant animal the interstitial cellular accumulation is more pronounced. Giant-cell formation, lymphocytic infiltration, vascularization of the tubercle, and fibrosis are more rapid and extensive in the resistant than in the susceptible animal.

The most impressive difference in the lesions observed in the two races is the acceleration in liquefaction which characterizes the development of the caseous focus of the resistant animal. Eight weeks after inhalation the extensively caseous masses of the tubercles in the susceptible animal which contain numerous bacilli (both isolated and in clumps) are compact and amorphous. In contrast, the corresponding foci in the resistant animal undergo rapid and extensive liquefaction. At the same time the few residual bacilli in this necrotic tissue may undergo massive proliferation. This results in cavities and, because of the free circulation existing between these foci and the rest of the body, a fresh flood of bacilli are carried to the draining lymph nodes. This is evidenced by the recovery of excessive numbers of bacilli from these nodes at this time, by the open lymphatics which are present in the walls of these cavities, and by the accumulation of erythrocytes in the lymph node sinuses. In the susceptible animal the large caseous foci, although still teeming with bacilli, remain largely closed off from the rest of the body because of the failure of the vascularization process to penetrate into these hard amorphous necrotic centers.

Discussion

An endeavor was made to elucidate the mechanism underlying the unique numerical and constant relations existing between the quantitative inhalation of bovine- and human-type tubercle bacilli, respectively, and the resulting formation of primary pulmonary foci in natively resistant and susceptible, highly

inbred rabbit races. An analysis has been made of the role of the virulence of the microorganisms concerned and a detailed study conducted of the fate of human-type bacilli at the portal of entry in the lung and their dissemination to the rest of the body. These two sets of observations of bacillary behavior have been correlated with the tissue responses in these contrasting races. It was thought that this type of comparative study might reveal some of the factors determining this varying resistance among the rabbit races, especially in the light of the newer knowledge of the effects of some of the adrenocortical hormones on inflammation and infection.

Previous studies indicated that, from an aerosol of highly virulent bovine bacilli, each microorganism which penetrates an alveolar space multiplies sufficiently to generate a tubercle in any part of the lung. This occurs irrespective of individual variations in virulence and without regard to the ecologic conditions (9) which might be conducive to the inhibition of its growth anywhere in the lung of a resistant or a susceptible host. The varied numbers of tubercles which result from the inhalation of a given number of human-type bacilli in resistant and susceptible animals (*see* Plate I, figures 1 and 2) at once bring into question the roles in tubercle formation played by the virulence of the individual bacillus which reaches a given site and the varying resistance of the tissues against the multiplication of the bacilli in different foci in these two races.

It was first demonstrated that the tuberculogenic capacity of the bacilli obtained either from a resistant or a susceptible host is of the same order when tested against a particular group of the same native resistance to the infection. Therefore, in these experiments, the individual virulence of human-type bacilli which reached the lung parenchyma of these rabbits played no significant role in the genesis of the tubercles produced in these two races. This was true from the standpoint of the number which is characteristic of each race of rabbit after inhalation of the same number of microorganisms.

A detailed study of the fate of human-type bacilli in the lungs of these races revealed that in both susceptible and resistant strains the population changes of the bacilli in their lungs were, in general, very similar to that of microorganisms which grow in an artificial medium. There was a lag phase in bacillary accumulation, a period of logarithmic growth, and a phase of decline. However, the data do not indicate whether this lag is due to the failure of growth of the bacilli in the tissues or to the balance between proliferation and destruction.

It was also evident from the very beginning of the infection and throughout its course that the lung of the susceptible animal was a more suitable medium for the growth of the bacilli than that of the resistant animal. At all intervals there was greater obstruction to the accumulation of bacilli in the lungs of the resistant than of the susceptible animal. From the first through the eighth week following inhalation, this inhibition was approximately twenty to thirty times greater in the resistant animal than in the susceptible animal. The greater initial inhibition to the multiplication of the bacilli in the resistant animals, by itself, accounts for the much longer lag period which precedes the logarithmic phase of growth in the resistant than in the susceptible animal. The longer lag

phase in the resistant animal was followed by a growth period. Therefore, this longer phase cannot be ascribed to a more rapid development of acquired resistance. It must be attributed to nonspecific constitutional factors which are inherent in the host from the very beginning of the infection.

It is these constant underlying nonspecific constitutional factors which condition, in a quantitative manner, the subsequent course of events. Both early during the growth phase and later, during the period of bacillary decline, when acquired resistance is apparently in force, the total pulmonary population changes remain twenty to thirty times more suppressed in the resistant than in the susceptible animal. It is clear, therefore, why acquired resistance is superimposed and determined by native resistance. It is also evident why cortisone, which diminishes the constitutional capacity of the cells to inhibit the accumulation of bacilli in their cytoplasm, can markedly reduce the acquired resistance induced by BCG vaccination (10).

The present study also indicates that a particular tubercle bacillus of human origin which is ingested by an alveolar phagocyte does not merely multiply or die, but may persist in the body and remain alive for considerable intervals without proliferating. This much greater inhibition to the multiplication of the bacilli in the lungs of the resistant animal accounts for the failure of many more bacilli which infest its macrophages to give rise to tubercle.

Furthermore, it was demonstrated that even those bacilli which succeed in multiplying in certain sites of the resistant animal do so to a much smaller degree than similar bacilli situated in phagocytes of the susceptible animals.

The fate of the bacilli in the lungs of the resistant and susceptible animals was correlated with the histologic responses in these two races. This revealed an impressive parallel existing between the relationship of a susceptible to a resistant animal, on the one hand, and that of a cortisone-treated to an untreated litter-mate of the same native resistance, on the other.

The intracellular accumulation of tubercle bacilli as early as two weeks after infection is much greater in the susceptible than in the resistant rabbit (*see* Plate II, figures 6 and 7). Cortisone markedly enhances the accumulation of the bacilli within the cells (4, 11). The maturation of the epithelioid cells is retarded in the susceptible as compared with the resistant animal (*see* Plate II, figures 8 and 9). Cortisone also retards the maturation of epithelioid cells (5). The progression of the caseous focus toward the amorphous state is delayed in the susceptible animal (*see* Plate III, figures 12 and 13). Cortisone also retards the maturation of the caseous process (12, 13). The pneumonic process is more pronounced than the intersitital inflammation in the susceptible rabbit (*see* Plate III, figures 10 and 11). Cortisone markedly suppresses the interstitial inflammation, and the lesion is dominated by foci of caseous pneumonia (11). Vascularization of the tubercle and the growth of fibrous tissue are slight in the susceptible animal and marked in the resistant animal (*see* Plate IV, figures 14 and 17). Cortisone suppresses the ingrowth of capillaries (12) and the formation of fibrous tissue (14).

Eight weeks after infection the extensive caseous foci in the susceptible ani-

mal are compact and little, if any, softening is found in these lesions (*see* Plate IV, figure 14). In the resistant animal the few residual lesions have undergone extensive liquefaction with the formation of grossly visible cavities (*see* Plate I, figures 4 and 5). It is noteworthy that softening is characteristic of a disease of long duration and that it is accelerated by immunization.

There is no information on the effect of cortisone on the softening process. It is evident, however, that in the susceptible animal this process is markedly retarded. As excess cortisone tends to hinder the maturation of many processes in tuberculous inflammation, it is possible that it may delay the development of softening also. As in a cortisone-treated rabbit, the bacilli in the lesions of the susceptible animal are much more numerous throughout the course of the disease than in the resistant rabbit. There is one exception to this generalization. During the process of liquefaction the bacilli may accumulate in certain disintegrating necrotic foci of the resistant animal in even greater numbers than are seen in the corresponding compact caseous centers of the susceptible rabbit (*see* Plate I, figure 5, and Plate IV, figure 15).

In regard to the dissemination of the bacilli from the portal of entry in the lungs to the draining nodes, a parallel similar to that seen above obtained between the relationship of a susceptible animal to a resistant, on the one hand, and of a cortisone-treated to an untreated rabbit, on the other. Contrary to all expectations, it was found that the bacilli from the pulmonary foci in the resistant animal are conveyed to the draining lymph nodes in much greater numbers than those in the susceptible animal.

It will be remembered that it was demonstrated in this laboratory that, with the adminstration of cortisone, the bacilli accumulate in massive numbers in the lung. Nevertheless, the draining lymph nodes in these rabbits contain far fewer bacilli than the nodes of an untreated litter-mate in whose lungs bacilli are scanty (11). Thus, cortisone retards the transport of bacilli from the focus to the lymph nodes. This was attributed to the suppression of inflammation and the reduction of vascular exchange between the focus and the rest of the body (5). A similar difference exists between the susceptible and resistant rabbits. In the former the vascularization of the tubercle and the accumulation of lymphocytes in the lymphatics are much less pronounced than in the resistant animal (*see* Plate IV, figures 14 and 16). Thus, in regard to exchange of cells and fluid between the focus and the rest of the body, the susceptible animal behaves as if it were under greater influence of cortisone than the resistant animal.

It must be stated in passing that the more rapid transport of the bacilli from the portal of entry to the draining lymph nodes in the natively resistant animal is entirely at variance with the once widely accepted view that the essential mechanism of acquired immunity is based on the mechanical or immunologic fixation of the bacilli at the site of reinfection. It was pointed out some years ago that the allergic inflammation in the immunized rabbit, far from fixing the bacilli at the site of reinfection, actually, because of the accompanying increased lymph pressure, sweeps them to the draining nodes more rapidly than in the normal individual (15).

As in the older studies on acquired immunity, so was it found in these studies

on native resistance. The failure of the disease to take root in the metastatic foci is due to the much greater inhibition to the multiplication of the bacilli at the foci of dissemination in the artificially immunized animal, on the one hand, and in the natively resistant animal, on the other.

The host-parasite relationships in the natively resistant and susceptible animals may likewise be scrutinized from the point of view of the adrenocortical hormone balance. It is tempting to suggest that many of the essential differences between these two races may be understood simply on the hypothesis that, in the presence of a tuberculous infection, the susceptible strain of animals is under greater influence of cortisone or its equivalent, hydrocortisone (compound F), than is the resistant animal. It is noteworthy that treatment of leukocytes with hydrocortisone can bring about a reduction in lactic acid production (16). The antiphlogistic influence of this hormone may also have the same effect (17). Dubos has demonstrated that lactic acid at low pH is bacteriostatic for tubercle bacilli (18). It is conceivable that by some such mechanism an increased hydrocortisone effect might enhance the multiplication of the bacilli in the tissues.

That the adrenocortical hormone balance is actually different in susceptible and resistant races is suggested by recent preliminary observations (19). The corticosteroids elaborated by the adrenals of the resistant race *III* used in these studies and those of another susceptible race, *FC*, were determined from the adrenal vein blood of these rabbits in the uninfected state, under the influence of corticotropin administration, and five weeks after inhalation of human-type tubercle bacilli. It was found that before infection both races produced largely corticosterone (compound B) and a small amount of hydrocortisone (compound F). However, the susceptible rabbits secreted twice as much compound F as the resistant animals. Under the influence of corticotropin, which tends to lower resistance to the disease (12), the elaboration of hydrocortisone is increased in both races, both relatively and absolutely. Five weeks after a tuberculous infection the susceptible animals did not increase their adrenal steroid output significantly over control levels. In the resistant tuberculous animals, however, the secretion of both F and B was increased.

Thus, there is suggestive evidence that constitutional factors influence steriodogenesis, which may correlate (to some degree) with genetic differences in resistance to tuberculosis.

Summary

The virulence of the individual cells of tubercle bacilli of human origin from an aerosol of H37Rv which penetrates the lungs of natively resistant and susceptible rabbits is not a significant factor in the genesis of the number of primary pulmonary tubercles which is characteristic for each race.

The bacillary population changes in the lungs of susceptible and resistant rabbits after quantitative inhalation of H37Rv, in general, resemble those of bacteria growing in an artificial medium. There is a lag phase in bacillary accumulation, a period of logarithmic growth, and one of decline. On this basis, the lung of the resistant animal is a poorer medium for the proliferation of the bacilli than the lung of the susceptible animal.

From the beginning of the tuberculous infection and through the eighth week, the inhibition of bacillary multiplication is twenty to thirty times greater in a resistant race of rabbits studied (*III*) than in a susceptible race (*C*). This differential inhibition by itself accounts for the longer lag phase which characterizes the growth of the tubercle bacilli in the resistant animal and the more rapid onset of logarithmic multiplication in the susceptible animal.

The greater initial inhibition of growth in the resistant animal is due to inherent nonspecific constitutional factors rather than to a more rapid development of acquired resistance.

Increased resistance to infection is acquired more effectively by the resistant race of rabbits. It is superimposed on, and quantitatively determined by, the native resistance.

Many of the bacilli that reach the lungs of both races remain alive for a considerable time without either dying or proliferating.

Because of the greater inhibition to multiplication of the bacilli in the lungs of the resistant animal, fewer of the surviving bacilli proliferate in its phagocytes and, thus, fewer tubercles are generated in this race than in the susceptible race of animals. The increased initial bacteriostasis of the resistant animal persists in the foci in which tubercles have formed.

The bacilli from the primary pulmonary foci in the resistant race are transported to the draining nodes in greater numbers than in the susceptible. More effective destruction of the focalized bacilli in the resistant animal rather than fixation of the microorganism at the portal of entry appears to be the mechanism which accounts for the failure of dissemination of the disease.

The host responses in the resistant race of rabbits are generally accelerated as compared with those in the susceptible animals, and are characterized by the following:

> (*a*) The bacillary accumulation within and outside cells is suppressed; (*b*) the maturation of the epithelioid cells and of the caseous focus is more rapid; (*c*) the interstitial inflammation is accentuated; (*d*) lymphocytic infiltration, giant-cell formation, vascularization, resorption of the tubercle, and fibroplasia are much more pronounced; (*e*) the exchange of cells and fluid between the pulmonary focus and the rest of the body is more active; (*f*) liquefaction and cavity formation, with their accompanying double-edged potentials for both eradication and spread of the disease, develop much faster in the resistant race.

Most of the differences in the behavior of the tubercle bacilli and in the host responses in the two races of rabbits are of the same nature as those between two rabbits of the same native resistance, one of which is under the intense influence of hydrocortisone and the other is not. However, the actual hormone constellation which controls resistance and susceptibility to the disease has not yet been determined.

Sumario

De la Naturaleza de la Resistencia Genética a la Tuberculosis a la Luz de las Relaciones de Huésped-Parásito en Conejos Naturalmente Resistentes y Susceptibles

La virulencia de los distintos bacilos de tipo humano procedentes de un aerosol de H37Rv, que penetran en los pulmones de conejos naturalmente resistentes y susceptibles no cons-

tituye un factor importante en la génesis del número de tubérculos pulmonares primarios, que es típico para cada raza.

Las modificaciones de la población bacilar en los pulmones de conejos susceptibles y resistentes, consecutivamente a la inhalación cuantitativa de H37Rv, semejan, en general, las de las bacterias cultivadas en un medio artificial. Hay una fase de retardo, un período de crecimiento logarítmico y otro de decadencia. Partiendo de esa base, el pulmón del animal resistente es un medio peor que el del animal susceptible para la proliferación de los bacilos.

Desde el comienzo de la infección y hasta la octava semana inclusive, la inhibición de la multiplicación bacilar es de veinte a treinta veces mayor en una raza resistente estudiada (HI) que en una susceptible (C). Esa inhibición diferencial explica de por sí la fase más larga de retardo que caracteriza el crecimiento de los bacilos en el animal resistente y la iniciación más rápida de la multiplicación logarítmica en el susceptible.

La mayor inhibición inicial del crecimiento en el animal resistente se debe a factores constitucionales anespecíficos inherentes más bien que a formación más rápida de resistencia adquirida.

La resistencia mayor es adquirida más rápidamente por la raza resistente; se sobrepone a la resistencia natural, la cual la determina cuantitativamente.

Muchos de los bacilos que llegan a los pulmones de ambas razas permanecen vivos por mucho tiempo sin morir ni proliferar.

Debido a la mayor inhibición de la multiplicación de los bacilos en los pulmones del animal resistente, un número menor de los bacilos sobrevivientes prolifera en los fagocitos y, por eso, se engendran menos tubérculos en esta raza que en la susceptible. La mayor bacteriostasis inicial del animal resistente persiste en los focos en que se han formado tubérculos.

Los bacilos procedentes de los focos pulmonares primarios de la raza resistente son transportados a los ganglios supurantes en mayores cantidades que en la raza susceptible. La destrucción más eficaz de los bacilos incluídos en los focos del animal resistente, más bien que la fijación de los microbios en la puerta de entrada, parece ser el mecanismo que explica la falta de diseminación de la enfermedad.

Comparadas con las de los animales susceptibles, se aceleran en general las respuestas del huésped en la raza resistente, caracterizándose por lo siguiente:

(*a*) se suprime la acumulación bacilar dentro y fuera de las células; (*b*) es más rápida la maduración de las células epitelioideas y del foco caseoso; (*c*) se acentúa la inflamación intersticial; (*d*) son mucho más destacadas la infiltración linfocítica, la formación de células gigantes, la vascularización, la reabsorción del tubérculo y la fibroplasia; (*e*) es más activo el intercambio de células y de líquido entre el foco pulmonar y el resto del organismo; y (*f*) en la raza resistente, se desarrollan mucho más rápidamente la licuación y la formación de cavernas con sus concomitantes potenciales de doble filo para la erradicación así como la difusión de la enfermedad.

La mayor parte de las diferencias en el comportamiento de los bacilos y en las respuestas del huésped en las dos razas es de la misma naturaleza de la que existe entre dos conejos de la misma resistencia natural, uno de los cuales se halla bajo el intenso influjo de la hidrocortisona y el otro no. Sin embargo, no se ha determinado exactamente la constelación hormónica que rige la resistencia y la susceptibilidad a la enfermedad.

Resume

De la nature de la résistance génétique à la tuberculose à la lumière des rapports hôte-parasite chez les lapins possédant une résistance ou une sensibilité naturelle

La virulence des bacilles individuelles de type humain d'un aérosol de H37Rv, pénétrant dans les poumons des lapins possédant une résistance ou une sensibilité naturelle, n'est pas un facteur significatif en ce qui concerne le nombre de tuberculomes pulmonaires primaires crées, qui est caractéristique pour chaque race.

Les modifications de la population bacillaire dans les poumons des lapins sensibles ou

résistants, après inhalation quantitative de H37Rv, ressemblent en général, à celles des bactéries cultivées en milieu artificiel. Il y a une phase ralentie, une période de croissance logarithmique et une de déclin. Sur cette base, le poumon de l'animal résistant est un milieu moins favorable à la prolifération des bacilles que celui de l'animal sensible.

Depuis le début de l'infection jusqu'à la huitième semaine incluse, l'inhibition de la multiplication bacillaire est de vingt à trente fois plus élevée dans une race résistante étudiée (III) que dans une race sensible (C). Cette inhibition différentielle en soi explique l'allongement de la phase ralentie, qui caractérise la croissance des bacilles chez l'animal résistant, et l'apparition plus rapide de la multiplication logarithmique chez l'animal sensible.

L'inhibition initiale plus marquée de la croissance chez l'animal résistant est due à des facteurs héréditaires non spécifiques plutôt qu'au développement plus rapide de la résistance acquise.

La résistance accrue est plus rapidement acquise par la race résistante; elle se superpose à la résistance naturelle qui la détermine quantitativement.

Un grand nombre des bacilles parvenant aux poumons, dans ces deux races, demeurent viables pendant un temps considérable sans mourir ou se reproduire.

En raison de l'inhibition plus marquée de la reproduction des bacilles dans le poumon de l'animal résistant, il y a moins de bacilles survivants proliférant dans ses phagocytes, donc il se produit moins de tuberculomes dans cette race que dans la race sensible. La bactériostase initiale accrue de l'animal résistant persiste dans les foyers où des tuberculomes se sont constitués.

Dans la race résistante, les bacilles des foyers pulmonaires primaires se portent vers les ganglions de drainage en plus grand nombre que dans la race sensible. Chez l'animal résistant, la destruction plus marquée des bacilles des foyers, plutôt que la fixation du germe au lieu d'entrée paraît être le mécanisme responsable de l'absence de dissémination de l'affection.

Par comparaison avec l'animal sensible, les réactions de l'hôte dans la race résistante sont généralement accélérées; elles sont caractérisées par:

(*a*) la réduction de l'accumulation bacillaire intra et extra-cellulaire (*b*) la maturation des cellules épithélioïdes et du foyer caséeux plus rapide; (*c*) la réaction inflammatoire interstitielle accentuée; (*d*) l'infiltration lymphocytaire, la formation de la cellule géante, la vascularisation, la résorption du tuberculome et la fibroplasie plus prononcées; (*e*) les échanges de cellules et de liquide entre le foyer pulmonaire et le reste de l'organisme plus actifs; (*f*) le ramollissement et l'excavation accompagnés de leur potentiel à double tranchant, éradication et dissémination, d'apparition beaucoup plus rapide dans la race résistante.

Dans les deux races, la plupart des différences dans le comportement des bacilles et dans les réactions de l'hôte sont de même nature que celles qui existent entre deux lapins possédant la même résistance naturelle, dont l'un est sous l'influence intense de l'hydrocortisone et l'autre qui ne l'est pas. Toutefois, la constellation hormonale existente qui contrôle la résistance et la sensibilité à la maladie n'a pas encore été déterminée.

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PLATE I

FIG. 1. The left lung of susceptible rabbit C 14=22, four weeks after the inhalation of an estimated 33,800 cells of *M. tuberculosis* H37Rv. Four hundred and seventy-one tubercles were present in the two lungs. Seventy-two inhaled bacilli were required to produce one pulmonary focus.

FIG. 2. The left lung of resistant rabbit HHR 5=3, exposed simultaneously with rabbit C 14=22 shown in figure 1 and killed at the same time after the inhalation of an estimated 41,750 bacilli of the same aerosol. Sixty-six smaller tubercles were present in the two lungs. Six hundred and thirty-three inhaled bacilli were required to produce one pulmonary focus.

FIG. 3. Section of lung of susceptible rabbit C 13=37, eight weeks after the quantitative inhalation of approximately 20,150 cells of *M. tuberculosis* H37Rv. Numerous large tubercles with solid caseous centers and extensive perifocal inflammation. No bronchogenic spread.

FIG. 4. Section of lung of resistant rabbit HH 6=24, exposed simultaneously with rabbit C 13=37 shown in figure 3 and killed at the same time, after the inhalation of approximately 26,350 bacilli. There is a well walled-off cavity indicated by the arrow in the isolated lesion above the main bronchus on the right and the pulmonary artery on the left, with bronchogenic spread toward the apex of the lobe.

FIG. 5. The septic membrane of the cavity wall of the liquefying lesion of HH 6=24 shown in figure 4. Numerous bacilli growing in the softening caseous tissue. (magnification × 538) The wall of this cavity is shown in figures 16 and 17 in Plate IV.

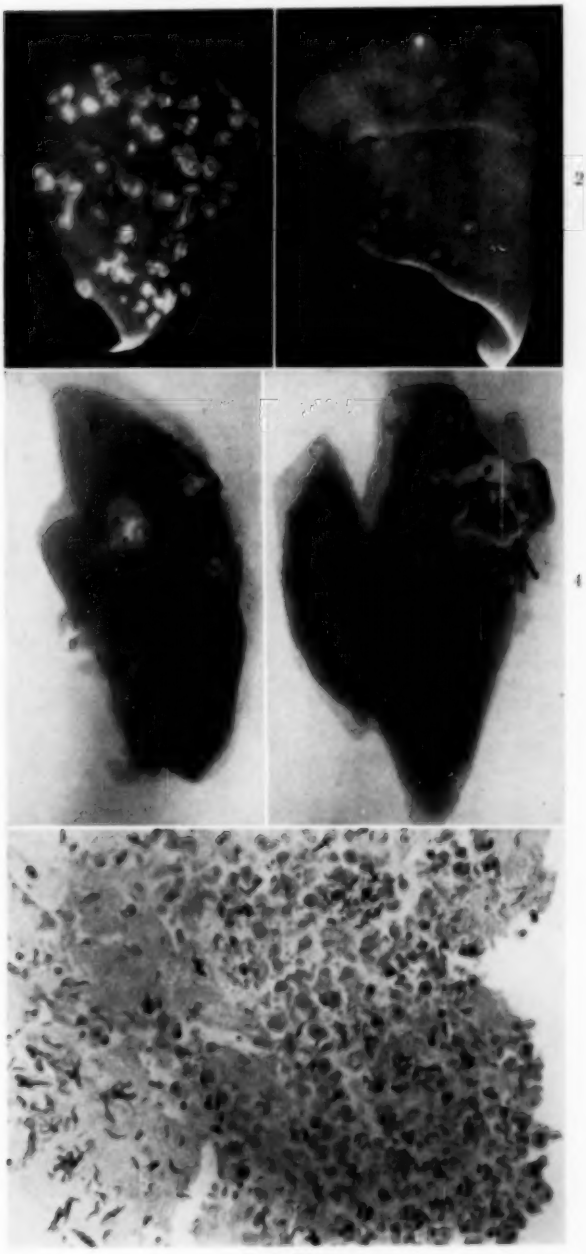

PLATE I

PLATE II

FIG. 6. Portion of an early tuberculous focus in the lung of susceptible rabbit C 14=5, two weeks after the quantitative inhalation of an estimated 35,400 tubercle bacilli. Numerous tubercle bacilli, many in the form of very dense skeins, swarm in the intra-alveolar phagocytes. Perivascular accumulation of mononuclears with some bacilli is seen in the lower left corner of the figure. There is little thickening of the surrounding alveolar septa. (magnification × 594)

FIG. 7. An early tuberculous focus in the lung of resistant rabbit IIIR 4=26, two weeks after the quantitative inhalation of 35,700 microorganisms. Moderate numbers of tubercle bacilli are seen in intra-alveolar phagocytes. Only one small skein, indicated by the arrow, can be seen in the lower alveolar space, which is infiltrated with numerous polymorphonuclear leukocytes. Interstitial thickening of the alveolar septa may be seen in the upper left hand corner of the figure. (magnification × 594)

FIG. 8. A tubercle in the lung of susceptible rabbit C 14=16, three weeks after the quantitative inhalation of 41,750 microorgasnisms. The edge of the caseous center of the tubercle, with numerous bacilli, may be seen in the lower right-hand corner of the figure. The surrounding immature epithelioid cells with reticulated undifferentiated cytoplasm and interconnecting processes contain numerous bacilli. (magnification × 594)

FIG. 9. A tubercle in the lung of resistant rabbit IIIR 5=2, three weeks after the quantitative inhalation of 39,700 microorganisms. A small focus undergoing caseation may be seen in the middle of the figure and on the left. Tubercle bacilli are rare. Numerous mature, rounded epithelioid cells with abundant ground-glass-appearing cytoplasm, differentiated by an inner light spherical zone and a darker peripheral ring, are seen showing few isolated bacilli. (magnification × 594)

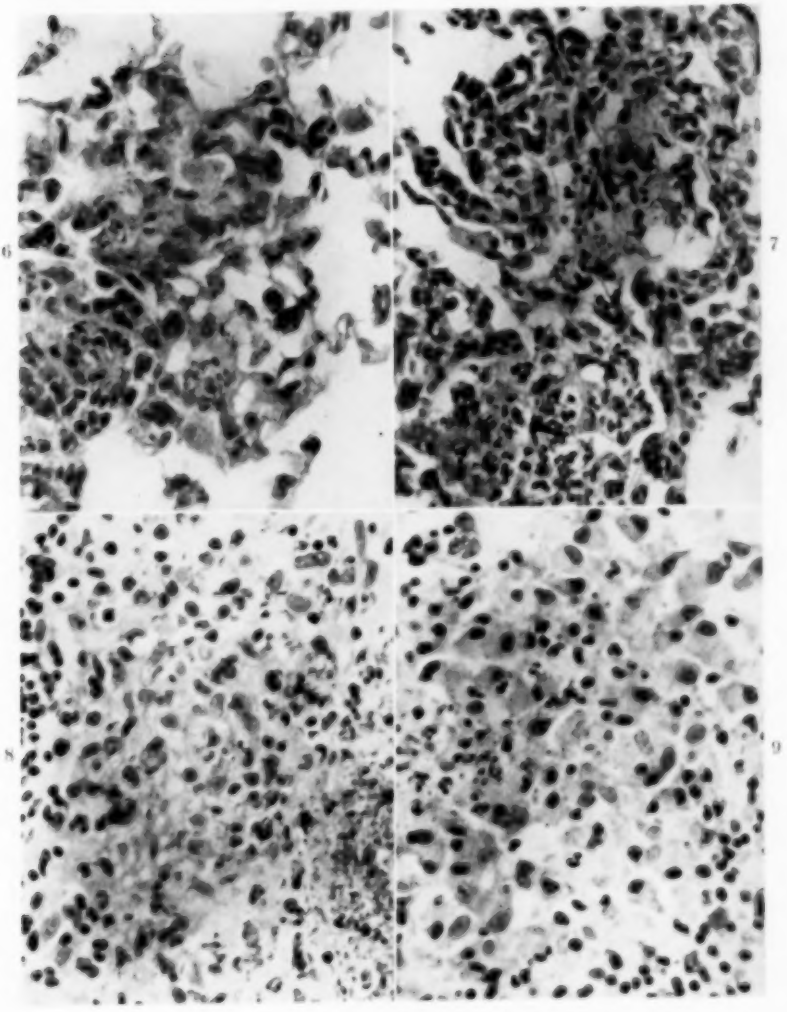

PLATE II

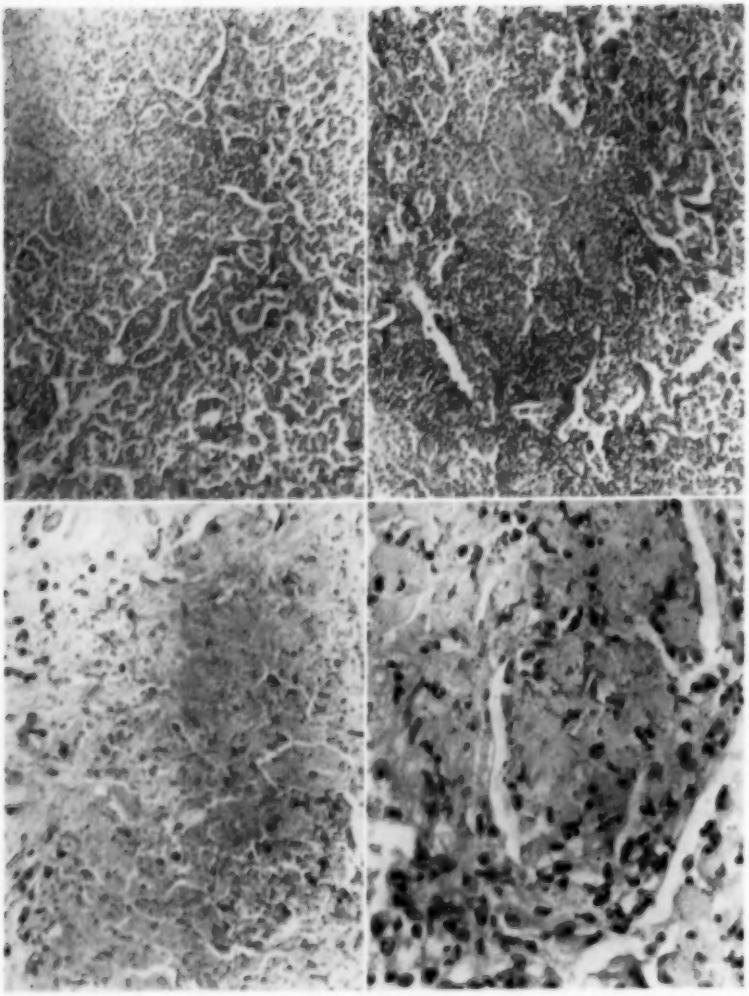

PLATE III

FIG. 10. A tuberculous focus in the lung of susceptible rabbit C 12=38, four weeks after the quantitative inhalation of 5,700 microorganisms. The edge of the caseous center in the upper left-hand corner and the prominence of the intra-alveolar exudative process are apparent. (magnification × 100)

FIG. 11. A tuberculous focus in the lung of resistant rabbit III 5=54, four weeks after the quantitative inhalation of 6,400 microorganisms. Slight caseation and prominence of the interstitial inflammatory process are apparent. (magnification × 100)

FIG. 12. Higher magnification of caseous focus from the susceptible rabbit C 12=38, part of which is shown in figure 10. Immature caseation with much residual nuclear debris and numerous bacilli are evident. (magnification × 538)

FIG. 13. Higher magnification of caseous focus from the resistant rabbit III 5=54, shown in figure 11. The caseation is more amorphous and mature and the bacilli are rare. (magnification × 538)

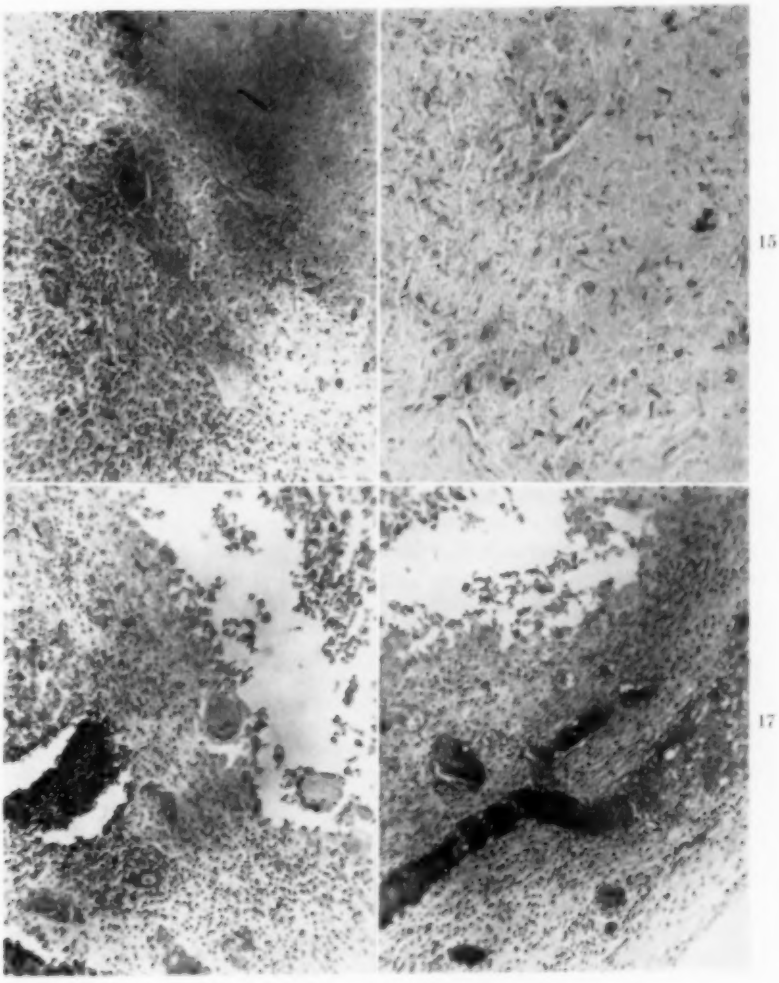

PLATE IV

FIG. 14. Tuberculous focus in susceptible rabbit C 13=2, eight weeks after the quantitative inhalation of 9,650 microorganisms. Large compact amorphous caseous center, surrounded by tuberculous granulation tissue, with some congested vessels. No fibroplasia. (magnification × 125)

FIG. 15. Higher magnification of portion of amorphous caseous focus of rabbit C 13=2 shown in figure 14. Numerous bacilli isolated and in clumps. (magnification × 558)

FIG. 16. The wall of a cavity in the lung of resistant rabbit HHR 5=4, eight weeks after the quantitative inhalation of 10,100 microorganisms. Massive accumulation of lymphocytes within a lymphatic vessel and giant cells in the membrane at the edge of the cavity. (magnification × 125)

FIG. 17. Another portion of the wall of the same cavity shown in figure 16. Fibroplasia and intense vascularization of the cavity wall are evident. (magnification × 125)

A SLIDE-CULTURE METHOD FOR TUBERCLE BACILLI IN THE LESIONS OF MICE[1, 2]

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(Received for publication January 20, 1955)

INTRODUCTION

Techniques have been devised and are now routinely used in some laboratories to grow tubercle bacilli directly in smeared sputum in contact with liquid medium (1–3). It seemed possible that the tubercle bacilli present in the spleens and lungs of mice infected intravenously could be grown in smears of the infected tissues in contact with a liquid medium by a slide-culture technique similar to that described for tuberculous sputum.

Indeed, by such a method, tubercle bacilli grow easily in typical serpentine colonies, and it has proved a convenient technique for research in the field of antituberculous agents as well as for diagnostic work and for current drug-susceptibility tests.

MATERIAL AND METHODS

The tuberculous lungs (or spleens) of the infected mice are ground in the presence of approximately 1 ml. of Dubos liquid medium or 0.5 per cent of bovine serum albumin fraction V[3] with a Teflon homogenizer, as described by Dubos and associates (4). One drop of emulsion is smeared at one end of ordinary glass slides cut in two longitudinally (so as to measure 12.5 by 75 mm.), covering approximately 2 cm. As many slides are thus prepared as are necessary for the planned drug-susceptibility tests, including several controls with no drugs.[4]

The slides are left to dry for a few minutes in a sterile Petri dish, transferred for six minutes in 6 per cent sulfuric acid, and finally washed in sterile water. (By proceeding under sterile conditions, an attempt was made to avoid treatment with sulfuric acid but, for some reason, the colonies have been much more scarce than on comparative slides treated with sulfuric acid.) It is convenient to place the slides on some sort of sterilizable holder on which they can be transferred simultaneously from one reagent to another. They are finally placed in 18 by 180 mm. test tubes containing 3 ml. of Dubos medium (with fraction V bovine albumin but with no Tween® 80) to which, when required, the antituberculous drug being studied has been added in serial dilutions.

The slide cultures are incubated at 37°C. After six to ten days, one of the control drug-free cultures is stained by the Ziehl-Neelsen technique and examined under the microscope.

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[2] This study was aided by grants from the Institut National d'Hygiène, Centre National de la Recherche Scientifique, and the Waksman Foundation.

[3] Grinding in the presence of 2 per cent of fraction V bovine albumin has proved much less satisfactory. The smear did not adhere as well to the slide and the culture was much poorer.

[4] In earlier experiments, impressions were made on the glass slide by pressure of the sectioned organ without previous grinding. The results were much less uniform.

If well-grown colonies are seen, all of the slides are then stained and examined under the low power of the microscope.

A culture of tubercle bacilli can usually be recovered from the remaining medium in which a well-grown slide culture was immersed. This makes it possible to check the results obtained with the slide-culture technique by the ordinary indirect drug-susceptibility tests.

RESULTS

It has been possible to obtain with regularity very typical "serpentine" colonies by slide cultures of the spleen or lung tissues. After four to ten days, these colonies are easily recognizable under low power, and the cellular bodies constituting these "cords" can be readily distinguished under the oil-immersion lens (figure 1 and figure 3 *B*).

If the smears are examined only after ten to fifteen days and if the specimen was heavily infected, a continuous reticular pattern is seen, in which the different colonies cannot be recognized and counted (figure 2, control (*A*) and isoniazid, 1 γ per ml. (*B*)). In contrast, when microscopy is done at an earlier stage, the enumeration of colonies is quite accurate, either per microscopic field or on the total preparation, which corresponds to a known volume of the organ suspension. When compared with the enumeration of viable bacilli by plating appropriate dilutions on Dubos agar medium, however, the colony counts have given values approximately two to three fold smaller (table 1).

In the drug-susceptibility tests made by slide culture, there is usually a clear-cut difference between the smears which have been in contact with completely inhibitory concentrations of a drug and those in contact with lower concentrations. Hence, the tests are easy to read. In other cases, some partial inhibitions are seen, as with 5 γ of isoniazid in the case represented in figure 2. It is worth noting that, in such cases of partial inhibition, the colonies are sometimes reduced not only in number but also in size.

No discrepancies have been found between the results of the drug-susceptibility tests with the slide-culture technique and with the routine indirect drug-sus-

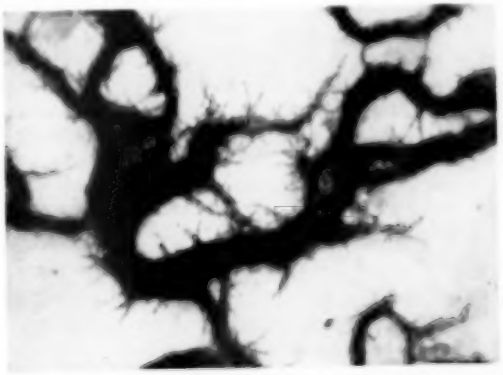

FIG. 1. Slide culture from the spleen of a mouse infected with H37Rv. (× 1,200)

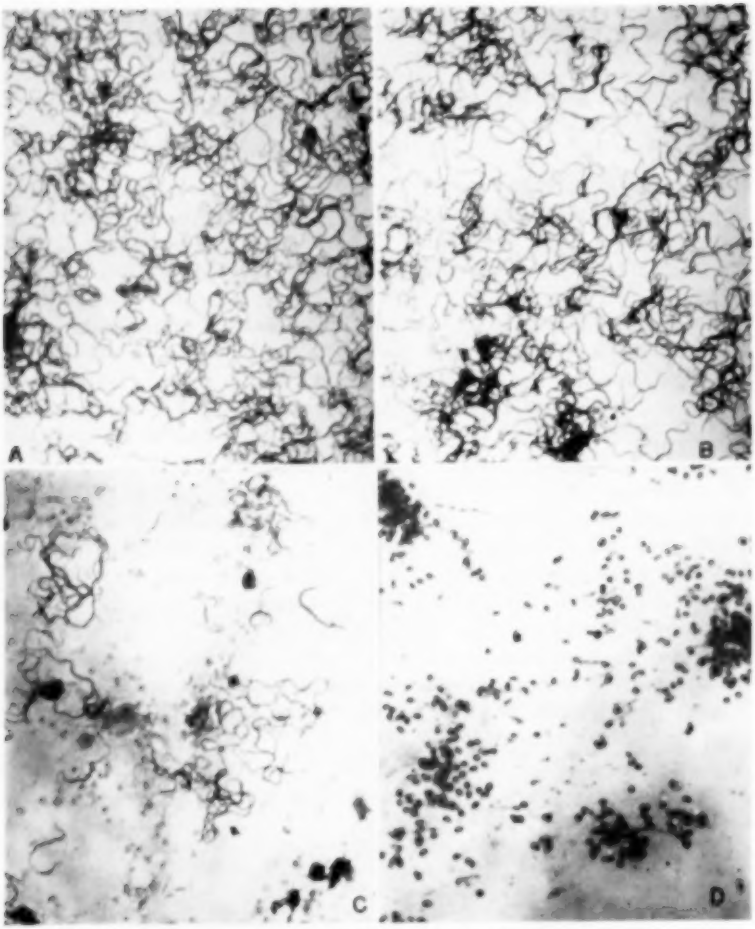

FIG. 2. Mouse infected with a strain resistant to isoniazid. Slide cultures from the spleen in the presence of 0γ (*A*), 1γ (*B*), 5γ (*C*), and 100γ (*D*) of isoniazid per ml. (× 160)

ceptibility tests made with the dilution method in Dubos medium with no Tween®.

Thus, the laboratory strains (H37Rv, Ravenel, Vallée) have been completely inhibited in most tests (12 of 15) by 0.03 or 0.06 γ of isoniazid per ml., which is their known range of drug susceptibility with any good method. In three experiments, 0.125 or 0.25 γ per ml. only have been inhibitory, but these strains had been recovered from mice treated with isoniazid.

With strains resistant to isoniazid, the results have been the same with both methods, as shown in table 1. The strains *D.*, *Leq.*, and *Bar.* were recovered

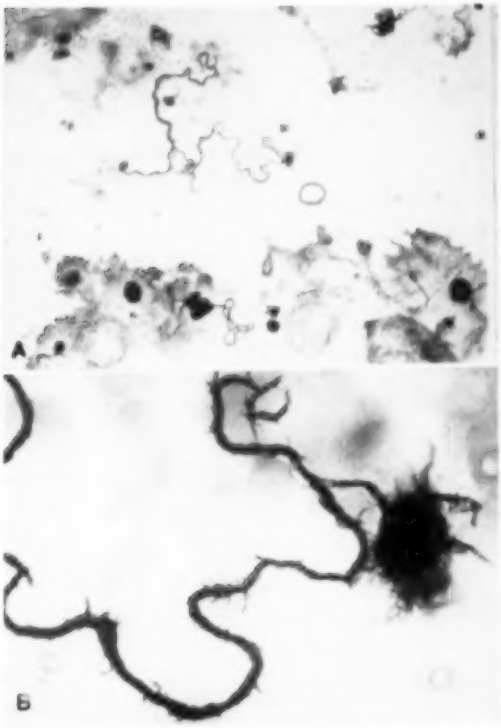

FIG. 3. Slide cultures from the spleen of a mouse killed two weeks after intravenous injection of cerebrospinal fluid from a patient with tuberculous meningitis. ((*A*) × 160; (*B*) × 1,280)

TABLE 1

COMPARISON OF ENUMERATIONS OF TUBERCLE BACILLI BY SLIDE CULTURE TECHNIQUE AND PLATING OF DILUTIONS ON AGAR MEDIUM

Cultures of Spleens of Mice Infected Intravenously with H37Rv (0.2 Ml. of 10^{-4} Dilution of Tween®-Albumin Culture) and Sacrificed Eight Days After Infection

Cultures of One Drop of Suspension of Spleen

	Slide Culture (Undiluted)	Dubos Agar Medium		
		Undiluted	1:10 Dilution	1:100 Dilution
1	279, 298, 158	Too numerous to count	64, 59, 57	4, 3, 7
2	190, 300, 453	Too numerous to count	57, 85, 88	3, 3, 5

TABLE 2

COMPARISON OF ISONIAZID SUSCEPTIBILITY TESTS WITH THE SLIDE-CULTURE METHOD AND WITH THE INDIRECT-DILUTION METHOD

	Isoniazid Concentrations (γ per ml.)											
	0	0.015	0.03	0.06	0.125	0.25	0.5	1	5	10	50	100
D.												
Slide culture	++++	++++	++++	++++	++++	++++	++	+	0	0	0	0
Indirect test	++++	++++	++++	++++	++++	++++	++++	+	0	0	0	0
Leq.												
Slide culture	++++						++++	+++	+	+	+	0
Indirect test	++++					++++	++++	+++	±	±	±	0
Bar.												
Slide culture	++	++			±			++		0		0
Indirect test					++	++	++	++	0	0	0	0
Ravenel isoniazid-resistant												
Slide culture	+++		+++	+	+++	+++	+++	+	+	+++	+++	+
Indirect test	++++		++++	++++	++++	++++	++++	++++	++++	+++	++	0

+++ and ++++: Continuous reticular pattern, more or less dense, but made of unseparated colonies.
++: Large and numerous colonies, separated one from another.
+: Smaller colonies, still seen on the whole smear.
±: Occasional colonies seen only on parts of the smear.

from sputum of patients treated with isoniazid; "Ravenel isoniazid-resistant" is the resistant mutant of the Ravenel bovine strain selected by subcultures in media containing increasing concentrations of isoniazid.

It may be seen in table 2 that the results with the slide-culture technique are sometimes much more irregular (Ravenel isoniazid-resistant) than with the usual indirect drug-susceptibility method. This would obviously be an important drawback, as it makes the reading of the limits of the inhibitory concentration somewhat doubtful. However, the results shown in table 2 were obtained with impressions of the sectioned organ. Smearing the emulsion of ground spleens or lungs has produced more regular results.

Comment: One of the main advantages of the slide-culture method used for drug-susceptibility tests is to avoid subcultures during which the initial drug susceptibility of the parental population can be altered. This is especially important for susceptibility tests to isoniazid, as resistance to this drug has conclusively been shown to be unstable through successive subcultures (5).

UTILIZATION OF THE METHOD

The method described above is now in routine use in this laboratory and has proved useful both in research work on antituberculous agents and in current clinical bacteriology.

Research Work on Antituberculous Agents

During treatment of experimental tuberculosis of mice, with either known antituberculous drugs or substances which have shown some activity in *in vitro* screening tests, it is always important to discover whether resistance has developed to the drug. Such observations are not always made routinely because it is time consuming and expensive to carry out the primary isolation of the bacilli from the tissues of the experimental animals, followed by subcultures and, finally, drug-susceptibility tests. The slide-culture technique makes the whole procedure much easier and quicker.

The method has also been useful in the study of virulence of tubercle bacilli resistant to isoniazid (6). It has been shown repeatedly that such bacilli have decreased pathogenicity for the guinea pig. In mice they produce disseminated lesions, but are less rapidly lethal than the drug-susceptible strains. The question arises whether less resistant variants in mixed populations are responsible for the widespread lesions. It is interesting, therefore, to study the susceptibility to isoniazid of the bacilli found in the lesions. Nevertheless, the results are always open to criticism if the organisms in the lesions are found less resistant than the injected organisms, as resistance to isoniazid has been shown to be unstable through subcultures.

Using the slide-culture technique, variants of relatively low virulence were found in the lesions for only one of six highly resistant strains which had been studied. This strain, isolated from a patient, had been the only one to show full virulence for the mouse. On the other hand, only highly resistant organisms were recovered from the less rapidly progressive lesions produced in mice by five other resistant strains.

The same slide-culture technique has been used in this laboratory to study the bactericidal action of isoniazid. The smears made from the spleens of infected mice were placed in media containing various concentrations of isoniazid and, after various lengths of time, transferred in media without drug. Whenever growth had taken place, ordinary drug-susceptibility tests were made on the organisms isolated from the remaining medium in order to find out whether they were drug-resistant variants or drug-susceptible organisms which had not been killed by isoniazid during the time of contact.

It should be pointed out that the use of the mouse in such experiments is no more than a means to get a well-dispersed suspension of tubercle bacilli which will adhere easily to glass slides. As a matter of fact, slide-culture techniques have been used already for this type of experiment by smearing cultures and not tissues of infected animals (7).

The usual difficulties, however, are: to smear cultures containing no clumps or serpentine colonies, so as to be sure that any clump or colony seen on the smear after contact with the medium really means that growth has taken place; to find a means of preventing the culture from falling into the liquid medium which allows at the same time unquestionable serpentine growth of the tubercle bacilli on the slides at the contact of the liquid medium.

Several methods have been used to avoid the pitfalls. Kreis sticks his cultures with egg white (7); Rubbo, with albumin solutions (3). In this laboratory both methods have been less easy and far less successful than the intravenous injection of a mouse and the smear of its ground tissues.

Clinical Routine Bacteriology

Rapid drug-susceptibility tests of tubercle bacilli previously isolated from pathologic specimens: In connection with rapid drug-susceptibility tests of strains from tissue specimens, the inoculation of the experimental animal has no other object than to give rapidly a well-dispersed suspension of the organisms which will adhere to the glass slides. As such, however, the present method can be useful when a quick answer is requested.

> For example, a very young culture on Löwenstein-Jensen medium was sent to this laboratory in order to find out as quickly as possible the susceptibility of the strain to isoniazid. The strain had been isolated from the gastric washings of a child with meningitis whose cerebrospinal fluid had yielded no tubercle bacilli and who was getting worse under treatment. A mixture of the still very small colonies was ground in the presence of Dubos medium with a Teflon homogenizer and the suspension was injected intravenously into two mice. Twenty-four hours later, the mice were killed and smears made from their spleens and lungs were put into medium with no drug and with serial dilutions of isoniazid. After six days, all of the cultures were easy to read and it was ascertained, just a week after the culture had been received, that this strain was resistant to 1γ of isoniazid per ml. and inhibited only by 10γ per ml. The indirect drug-susceptibility test subsequently confirmed this result. Moreover, the validity of both tests was likewise confirmed by the fatal evolution of the meningitis in spite of treatment.

Primary isolation of tubercle bacilli from pathologic specimens with simultaneous drug-susceptibility tests: Perhaps the most interesting utilization of the method

is for direct drug-susceptibility tests on primary isolations of tubercle bacilli. Indeed, this role might prove the most useful one for the present method, although it is not yet adapted to widespread use.

It is to be hoped that tubercle bacilli injected intravenously, even in rather small numbers, would multiply in the organs of the mouse rapidly enough to be detectable in the tissues by the slide-culture method long before visible lesions had time to develop.

Indeed, Dubos and associates (4) have shown, by plating the ground tissues of mice infected intravenously with diluted cultures on agar medium, that tubercle bacilli could be found in all of the organs twenty-four hours after infection and that, from that time on, their number increased in the spleen, reaching a maximum two to three weeks later.

A similar experiment was made with the slide-culture technique instead of the enumeration by cultures on agar medium.

Albino mice, four weeks old, were infected intravenously with 0.2 ml. of a well-grown 10-day-old culture of H37Rv in Dubos Tween[3] albumin medium diluted to 10^{-3} for half of the mice and to 10^{-4} for the other half. Enumerations on agar medium were to show that each mouse had received approximately 8,000 (10^{-2}) or 800 (10^{-3}) viable units of tubercle bacilli.

The mice were killed one, two, eight, and seventeen days after infection. Impressions were made on slides with the spleens and the lungs. Then the organs were ground and smears were made of the suspensions. For each organ, one slide with the impression and one with the suspension were immediately stained and examined; all of the others were placed in Dubos liquid medium and examined ten days later.

The results are given in table 3, in which it may be seen that:

1. By the slide-culture method, organisms are detected which could not be seen by direct examination under the oil immersion.
2. With the smaller infective dose (10^{-3}), numerous tubercle bacilli could readily be found in the spleens after eight days and were greatly increased after seventeen days. One or two days after infection, they could only occasionally be detected. With the largest infective dose (10^{-2}), the organisms were found after one and two days. They had multiplied extensively after eight days and even more after seventeen days.
3. In the lungs, after the same length of time, many fewer organisms were found.

It could be hoped, therefore, by injecting a specimen of cerebrospinal fluid from a patient with tuberculous meningitis intravenously into mice, for instance, that the organism could be quickly discovered and at the same time tested for its susceptibility to the antituberculous agents.

The present method has not yet been tried on a large scale. However, it gave interesting and encouraging results in a test conducted upon the cerebrospinal fluid of a child who had tuberculous meningitis as yet untreated. Numerous colonies were seen in the control culture (figure 3) and, exactly sixteen days after the initial lumbar puncture, it was possible to state that this strain was normally susceptible to streptomycin and isoniazid.

TABLE 3

MICE INFECTED INTRAVENOUSLY WITH DILUTED CULTURES OF H37Rv AND SACRIFICED AT VARIOUS INTERVALS AFTER INFECTION
Slide Cultures of Spleens and Lungs

Mice Killed After:		Mice Infected with Culture Diluted to 10^{-5}				Mice Infected with Culture Diluted to 10^{-6}			
		Spleen		Lungs		Spleen		Lungs	
		Direct Examination	Slide Culture	Direct Examination	Slide Culture	Direct Examination	Slide Culture	Direct Examination	Slide Culture
days									
1	Impression	0	1 colony	0		0	0	0	
	Suspension	0	5, 4, 5	0	0, 1, 3	0	1	0	0
2	Impression	0	2, 5	0	0	0	0, 0, 0	0	
	Suspension	0	4, 3, 2	0	1, 0, 0	0	0, 0, 0	0	0, 0, 0
8	Impression	0	++, ++, ++	0	4, 1	0	1, 0, 7	0	0, 10
	Suspension	0	++, +++, +++	0	11, 20, 21	0	30, ++	0	0, 0, 0
17	Impression	+	++++, ++++	+	More than 20	+	++++, ++++	0	0, 0, 0
	Suspension	+		0	0, 0, 0	+		0	0

The figures indicate the number of colonies found on the whole smear and correspond to
+ : Less than 1 colony per field
++ : 1 to 5 colonies per field
+++ : 5 to 10 colonies per field
++++ : More than 10 colonies per field.

These clinical utilizations of the slide-culture method are now being tried on a more widespread basis.

SUMMARY

A slide-culture method is described for suspensions of tissues of mice infected with tubercle bacilli. Different possible utilizations of this method for research, diagnosis, and drug susceptibility tests are presented.

SUMARIO

Método para el Cultivo en Laminillas de los Bacilos Tuberculosos en las Lesiones de los Ratones

Describese un método de cultivo en laminillas para las suspensiones de tejido de ratones infectado con bacilos tuberculosos. Menciónanse distintas posibles utilizaciones de este método para la investigación, el diagnóstico y pruebas de la fármacosusceptibilidad.

RESUME

Méthode de la culture sur lame des bacilles tuberculeux dans les lésions de la souris

Description d'une méthode de culture sur lame de suspensions de tissus prélevés chez la souris infectée par le bacille tuberculeux. Présentation de différentes applications possibles de la méthode pour la recherche, le diagnostic et les tests de sensibilité aux médicaments.

Acknowledgment

The writers are indebted to Mlle. Kolsky for the photographs in the present study.

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EFFECT OF BCG VACCINATION ON FREE FATTY ACID AND TRIBUTYRINASE CONTENT OF RABBIT TISSUES[1]

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(Received for publication February 21, 1955)

INTRODUCTION

Although there appears to be rather convincing evidence that BCG vaccination affords at least some measure of immunity to tuberculosis, the exact mechanism involved is not understood. In view of the marked toxicity for tubercle bacilli of even minute traces of certain naturally occurring fatty acids (1–6), it was thought to be of interest to determine whether BCG vaccination results in any significant increase in the free fatty acid content of animal tissues.

In 1916, Porter (7) postulated that, since fatty acids are toxic for tubercle bacilli, those tissues rich in esterases should be resistant to infection. In a preliminary study on the distribution of esterases in the organs of a wide variety of different animal species, the distribution of butyrinase and lecithinase appeared to have some relation to resistance to tuberculosis. The cat, which is resistant to tuberculosis, was well provided with esterases, while the susceptible guinea pig was deficient in these enzymes. The lungs of all species were very poor in esterases. In a later study, Porter (8) showed that there was a rather consistent relationship between lipolytic activity and bactericidal action on tubercle bacilli *in vitro*. Along somewhat similar lines, Gerstl and Tennant (9) showed that tuberculophospholipid was split more rapidly *in vitro* by enzymes from the resistant mouse than by those from the more susceptible rabbit and guinea pig. Guinea pig lung did not split the phospholipid at all in any of the experiments. In the light of these findings, there has been included in the present investigation a study to determine whether BCG vaccination increases to any degree the lipolytic activity of animal tissue.

MATERIAL AND METHODS

Protective effect of BCG vaccination in rabbits: In a preliminary study the protective effect of BCG vaccination in rabbits was measured in order to determine the proper dosage and time relationships upon which to base the fatty acid and enzyme determinations. Three albino rabbits, each weighing approximately 1,500 gm., were given 15 mg. (wet weight) of BCG[2] organisms intravenously in the marginal ear vein. Two months later the vaccinated animals, together with 4 nonvaccinated control animals, were each challenged intravenously with 0.1 mg. (wet weight) of a virulent bovine Ravenel[3] strain. The nonvaccinated rabbits died in 24 to 94 days (average survival, 45 days); the vaccinated animals, in 101 to 112 days (average survival, 105 days). The only gross evidence of disease consisted of pulmo-

[1] From the Tuberculosis Research Laboratory, Veterans Administration Hospital, Washington, D. C.

[2] Original culture obtained from Dr. S. R. Rosenthal, Tice Laboratory, University of Illinois, Chicago, Illinois.

[3] Obtained from Dr. William Steenken, Jr., Standard Culture Depot, Trudeau, New York.

nary lesions and these were more extensive in the control animals than in the vaccinated rabbits.

BCG vaccination and collection of tissues: On the basis of the findings just described, 3 albino rabbits (1,500 gm.) were given 15 mg. of BCG organisms intravenously. At the end of two months these animals, together with 3 nonvaccinated rabbits, were fasted overnight and killed by occipital trauma. Lungs and livers were removed, washed free of superficial blood with distilled water, immediately frozen at −20°C., and stored at that temperature until analyzed, usually within a few hours.

Free fatty acid determination: Approximately 0.5 gm. samples of tissue were cut from the frozen organs and immersed in 25 ml. of hot (boiling water bath) freshly distilled 95 per cent ethyl alcohol for fifteen minutes. The tissue was then transferred to a mortar and ground thoroughly with gradual addition of the alcohol in which it had been heated. The homogenate was then transferred back to the original tube. The mortar was rinsed out three times with 10 ml. of alcohol and these washings were pooled with the tissue homogenate. After centrifugation at 3,000 r.p.m. for fifteen minutes, the tissue residue was dried to constant weight at 70°C. and the supernatant was evaporated to dryness with gentle heat. Free fatty acids were isolated from the alcoholic residues by the technique described by Fairbairn (10) and titrated with fiftieth-normal sodium hydroxide using 2 drops of either 1 per cent alcoholic phenolphthalein (lung) or 1 per cent alpha naphthol benzine (liver) as an indicator. Results were recorded as milliliter of fiftieth normal sodium hydroxide per gram of dried tissue.

Tributyrinase determination: Samples of frozen tissue were weighed and homogenized for one and one-half minutes in a Waring Blendor with 8 volumes of physiologic saline. The tissue homogenate was frozen at −20°C. for two hours, thawed, centrifuged at 3,000 r.p.m. for forty five minutes, and the supernatant was decanted and used as enzyme. The substrate consisted of an aqueous tributyrin suspension (100 mg. per ml.) in 5 per cent gum acacia. Sodium diethylbarbiturate (0.1 M), adjusted to pH 8.6 with 0.1 M hydrochloric acid, was used as a buffer. Experimental tubes were prepared by combining 1 ml. of enzyme solution, 1 ml. of tributyrin, and 1 ml. of buffer. Controls were the same except that 1 ml. of water was used instead of enzyme. All tubes were incubated at 37°C. for one hour and the reaction was stopped by the addition of 10 ml. of freshly distilled 95 per cent ethyl alcohol. After centrifugation at 3,000 r.p.m. for fifteen minutes, the supernatant fluid was titrated to pH 10.0 with fiftieth normal sodium hydroxide in a Coleman Model 20 Electrometer. Results obtained with the controls were subtracted from the titration values of the experimental tubes.

RESULTS

It may be seen from table 1 that BCG vaccination in rabbits produced an increase in the free fatty acid content in the lung and a decrease in the liver.

TABLE 1

EFFECT OF BCG VACCINATION ON FREE FATTY ACID CONTENT OF RABBIT TISSUES

	Milliliter of N/50 Sodium Hydroxide Per Gram of Dry Tissue			
	Lung		Liver	
	BCG	Control	BCG	Control
Number of determinations	12	12	10	12
Mean	2.13	1.50	2.00	2.50
Standard error of mean	0.218		0.177	
t value	2.89*		2.82*	

* Statistically significant at 0.01 probability level.

TABLE 2

EFFECT OF BCG VACCINATION ON TRIBUTYRINASE CONTENT OF RABBIT TISSUES

	Milliliter of N/50 Sodium Hydroxide			
	Lung		Liver	
	BCG	Control	BCG	Control
Number of determinations	18	18	17	18
Mean	3.95	3.52	10.65	10.30
Standard error of mean	0.19		0.44	
t value	2.26		0.79	

These differences were statistically significant. The results presented in table 2 indicate that slightly higher tributyrinase concentrations were obtained in the lungs and liver of the BCG-vaccinated animals as compared with the controls; however, the differences were not statistically significant at the 0.01 probability level.

DISCUSSION

It would obviously be premature to assume that there is a causal relationship between the protective effect of BCG vaccination in rabbits, as measured by increased survival time after challenge with virulent organisms, and the elevated content of free fatty acid in the lung which apparently resulted from the vaccination. However, certain findings of the study seem worthy of further discussion.

Any interpretation of the results presented must take into account the fact that a comparatively large amount of vaccine was used (10 mg. per kg.). This is in excess of the dosage ordinarily used in vaccination of humans. Moreover, the vaccine was administered intravenously rather than by the usual intracutaneous technique. It is of interest to note, however, that even under these conditions the vaccination was capable of prolonging survival time by an average of only sixty days and did not prevent the development of pulmonary disease. In view of these facts it should be emphasized that, even though it may eventually be established that BCG vaccination protects rabbits by increasing the free fatty acid content of certain tissues, it does not necessarily follow that the same mechanism applies in other animals or in man. Presumptive evidence has been obtained in the present laboratory which suggests that this mechanism apparently does not operate in the guinea pig (11).

From the present results it appears that the most susceptible organ, the lung, had a lower normal concentration of free fatty acid than did the more resistant liver. This is true of certain amino acids as well, and these amino acids have also been shown to be elevated in the lungs of BCG-vaccinated rabbits (12). It is difficult to explain the decrease in the free fatty acid concentration of the liver in the vaccinated animals. It is interesting that, despite this decrease, the concentration was still of the same order as that in the lungs of the vaccinated rabbits.

It has been suggested that fatty acids do not necessarily exist free in living tissues and that their presence may be due to the autolytic decomposition of cell lipids during the preparation for analysis. Using techniques designed to minimize autolysis, Fairbairn (10) found that the free fatty acid content of whole mice was very small (2.2 mg. per gm. dry, fat-free tissue). He considered that even this low value might be too high, since freezing of the tissues was not instantaneous. An effort was made in the present study to control autolysis by freezing and storing the tissues at −20°C. and by treating them within a few hours after collection. While the values reported for free fatty acid in the present study may also be in excess of true concentrations, the conclusions drawn would seem to be valid as it is the relative values which are of principal interest.

It is of further interest to note that, as with the free fatty acids, the more susceptible lung had a lower normal concentration of tributyrinase than did the more resistant liver. Whatever mechanism may be involved in the alteration in free fatty acid concentrations following BCG vaccination, it does not appear to be related to lipolytic activity in the lung or liver. Although slightly higher values for tributyrinase were observed in BCG-vaccinated rabbits than in control animals, the differences were not statistically significant. In contrast, the tributyrinase activity of guinea pig organs has been reported to be significantly higher in BCG-vaccinated animals than in controls (13). This may be a reflection of differences in technique or of an actual species difference.

SUMMARY

Vaccination of rabbits with BCG results in a statistically significant increase in the free fatty acid content of the lungs and a decrease in the liver. No significant alteration in the tributyrinase activity of the lung or liver could be detected.

SUMARIO

Efecto de la Vacunación con BCG sobre el Contenido de Acido Graso Libre y de Tributirinasa en los Tejidos del Conejo

La vacunación de los conejos con BCG da por resultado un aumento estadísticamente importante en el contenido de ácido graso libre en el pulmón y una disminución en el hígado. No pudo descubrirse ninguna alteración importante de la actividad de la tributirinasa en el pulmón o el hígado.

RESUME

Effet de la vaccination par le BCG sur la teneur en acide gras libre et en tributyrinase des organes du lapin

La vaccination par le BCG provoque l'augmentation statistiquement significative de la teneur en acide gras libre du poumon et sa diminution dans le foie. Aucune altération significative de l'activité de la tributyrinase du poumon ou du foie n'a pu être décelée.

Acknowledgment

The writers wish to thank Dr. Carleton R. Treadwell, Department of Biochemistry, George Washington University School of Medicine, for his helpful suggestions regarding certain technical details involved in this study.

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DETECTION OF APPARENTLY ABSENT CIRCULATING ANTIBODIES IN TUBERCULOUS SERA[1,2,3]

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(Received for publication January 10, 1955)

INTRODUCTION

The absence of detectable circulating antibodies in a substantial number of patients with active tuberculosis has been a major factor in preventing the clinical use of any of the serologic tests in the diagnosis of tuberculosis. This holds true for the complement-fixation test (1), precipitation and agglutination reactions (2), and their modification (3); the Middlebrook-Dubos test (4–8) and its modifications (9).

The relatively large number of tuberculous patients without manifest circulating antibodies is remarkable when compared with the nearly 100 per cent positive response to tuberculin skin tests, or with the incidence of circulating antibodies in other granulomatous diseases, e.g., coccidioidomycosis (10) or brucellosis. This is also of theoretical interest in view of the demonstrated ability of tubercle bacilli to enhance the production of antibodies to other antigens (11).

The absence of circulating antibodies may be only apparent, for it is conceivable that inadequate sensitivity of the procedure employed, the presence of inhibiting substances, incompleteness of antibody, or its *in vivo* neutralization may be responsible for the inability to detect the antibodies.

Attempts to enhance the sensitivity of the Middlebrook-Dubos test have been reported (6, 7). A procedure devised in the present laboratory (7), similar to that employed later by Coombs and co-workers (12) for detecting incomplete or nonprecipitating antibodies, yielded only 81 per cent positive results in a group of 124 patients (13). Hall and Manion's modification (6), adding the technique of the indirect Coombs' test, did not yield positive tests in sera which failed to react in the original Middlebrook-Dubos procedure (13).

The observation that lymphocytes produce or carry antibodies and that diminution of lymphocytes after injection of corticosterone is associated with a marked rise in antibody titer (14) suggested the possibility that the absence of circulating antibodies might be due to their retention by the leukocytes. Accordingly, this problem was investigated, but no clue to the cause for the absence of antibodies in these patients was found. The presence of an inhibiting factor in the serum was assumed (15), as suggested by the studies of Cann and

[1] From the Veterans Administration Hospital, Oakland, California.

[2] This investigation was supported by the U. S. Veterans Administration and by grants from the Committee on Medical Research of the American Trudeau Society, medical section of the National Tuberculosis Association; and from the California Tuberculosis and Health Association.

[3] Presented before the Medical Session, as part of *Section 4B*, at the annual meeting of the National Tuberculosis Association, Milwaukee, Wisconsin, May 24, 1955.

co-workers (16). Studies directed to this question form the basis of the present report.

MATERIALS

The tuberculous sera were obtained from patients with active tuberculosis who were admitted to the tuberculosis ward of this hospital. The diagnosis was established clinically and confirmed bacteriologically. The National Tuberculosis Association's criteria (17) were used for classifying the activity and extent of the pulmonary disease. The control sera were collected from patients whose diagnoses are listed in table 3. All sera were stored in the refrigerator for twenty-four hours before use.

METHODS

Estimation of circulating antibodies: The hemolytic modification of the Middlebrook-Dubos test (18) was employed. This procedure was carefully followed. Old Tuberculin, four times the usual strength, Lederle[4] Lot 33, was used for sensitizing the erythrocytes.

The complement-fixation test was carried out, using Raffel's (19) technique.[5]

Elution of antibodies from leukocytes: Two procedures were employed. In one (20), heparinized blood was placed in plastic tubes and allowed to sediment while kept at an angle of 60 degrees for ten minutes at room temperature. Then the tubes were centrifuged at 2,500 r.p.m. for thirty minutes, and rapidly frozen. Erythrocytes, leukocyte layer, and plasma were separated by cutting the plastic tubes with a razor. The second method was to collect blood with ammonium-potassium oxalate as anticoagulant, centrifuge the blood, and remove the plasma. The buffy coat was transferred to a 6 mm. tube by means of a capillary pipette and centrifuged again. The leukocyte layer at this point was relatively large in comparison with the erythrocyte layer and could be removed without any erythrocytes adhering.

The leukocytes recovered from 10 ml. of blood were suspended in 1 ml. of plasma of the same patient and disrupted by freezing and thawing three times. Then, the suspension was centrifuged to remove any particulate matter, and the supernatant was used in the Middlebrook-Dubos test. In another experiment the reaction mixture contained 1.2 ml. of plasma, the buffy coat, 0.3 ml. of saline, and 0.1 ml. of an antimicrobial drug mixture (120 units of sodium penicillin G and 600 mg. of streptomycin per ml. dissolved in 0.1 per cent glucose). After freezing and thawing three times, this was incubated for six hours at 37°C. in a roller tube. The cell fragments were then separated by centrifugation, and the supernatant was used in the Middlebrook-Dubos test.

Fractionation of serum proteins: The cold ethanol method, as recommended by Nichol and Deutsch (21), was employed. This procedure had been designed for the separation of gamma globulins, leaving complexes of various proteins in the other fractions. The latter, labeled according to Nichol and Deutsch, are: *supernatant A*, largely albumin; *precipitate B*, largely beta and gamma$_1$ globulins; *precipitate C$_2$*, gamma$_2$ globulin. The fractions, after lyophilization, were kept at −15°C. By this method antibody activity was preserved, but it was noted that repeated thawing and freezing rapidly reduced antibody activity. Serum specimens Nos. 1, 2, 3, 4, 9, 10, and 11 were fractionated in a cold bath, all others in a cold room. The residue, after lyophilization, was taken up in an amount of buffered saline (18) either equal to or less than that of the serum. The degree of concentration is indicated in tables 1, 2, and 3.

Characterization of protein fractions: Paper electrophoresis, as recommended by Grassmann and Hannig (22), was carried out with a veronal buffer of pH 8.6, 0.1 ionic strength, with a potential of 1.3 volts per cm. of length of strip, and a current of approximately one

[4] Tuberculin was generously supplied by Dr. Piersma of Lederle Laboratories, Pearl River, New York.

[5] Purified tuberculoprotein was kindly and generously supplied by Dr. S. Raffel.

milliampere per cm. of width. The electrograms were stained with bromphenol blue (23). Densitometry was performed on a Beckman DU spectrophotometer at 575 millimicra wave length. Transparency of the strips was increased by first immersing them in a paraffin oil-alpha-bromnaphthalene mixture with a refractory index of 1.51. An aliquot of the original serum was run on the same strip. Four to 20 microliters of the solution were placed upon the paper, the amount depending upon the protein content. The latter was determined by the micro-Kjeldahl procedure.

The electrograms of whole serum showed a pattern similar to that reported by Grassmann and Hannig. When fractions obtained by the ethanol procedure were placed on the filter paper, a small portion of the protein sometimes precipitated, forming a round spot 2 to 3 mm. in diameter at the point of origin. In the electrogram this is represented by a peak which is considerably narrower than that due to gamma globulins.

RESULTS

Elution of antibodies from leukocytes: The first modification of the procedure outlined was applied to 7 specimens from patients with active pulmonary tuberculosis. Three of the patients had not shown circulating antibodies in the hemagglutination test. The other 4 subjects (controls) had an antibody titer of 1:128. The second procedure was employed on specimens of 4 tuberculous patients, 3 of whom failed to exhibit humoral antibody activity; the fourth one was used as a control. Antibodies could not be demonstrated in any of the samples of the negative reactors. The controls showed antibody activity of the same titer, or onefold dilution less than the original serum.

Fractionation of serum proteins: In table 1 are given the serologic results with protein fractions obtained from the 8 sera which reacted in the Middlebrook-Dubos test.

As the gamma globulins are considered to be the carriers of antibody activity, *precipitate* C_2, which contains the major part of the antibodies, is discussed first. Each precipitate C_2 of the 8 sera showed antibody activity in the Middlebrook-Dubos test, as expected, and in the 6 instances in which a complement-fixation test was done. Separation in terms of antibody activity was complete only in serum No. 4, although the presence of gamma globulins in supernatant A and precipitate B was suggested by the electrogram. All three fractions of the remaining sera of this group contained antibodies in varying amounts. Fraction A of sera Nos. 2 and 6 showed a hemagglutinating titer comparable with that of the corresponding precipitate C_2, but did not react in the complement-fixation test. Electrograms of these fractions revealed that small amounts of slow-moving globulins were present. The latter, calculated on the basis of planimetry of the electrophoretic curves and of total nitrogen, contained 43.7 and 53.4 mg. of nitrogen per 100 ml., respectively. This amount is greater, for instance, than that present in fraction C_2 of serum No. 2 and could account for the antibody activity.

Fraction B of serum No. 8 showed hemagglutinating activity which, in terms of titer, was a multiple of that of fraction C_2. The complement-fixing activity, however, was more concentrated in precipitate C_2. Serum No. 5, which failed to react in the complement-fixation test, yielded a precipitate C_2 which did react.

After it had been demonstrated that the fractionation procedure does not

TABLE 1

Serologic Results on Fractions of Tuberculous Sera Reacting in Hemagglutination Test

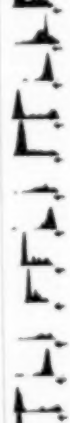
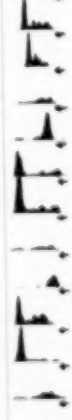
Serum Number	Diagnosis	Serum Fraction	Titer of Test		Concentration	Nitrogen in Mg. per 100 Ml.	Electrophoretic Pattern
			Hemagglutination	Complement Fixation			
1	Tuberculous enterocolitis, peritonitis, and lymphadenitis	Whole serum	1:160	±		1,008	
		Supernatant A	1:8	Negative	1×	930	
		Precipitate B	Negative	±	1×	143	
		Precipitate C_1	1:32	>1:32	1×	100	
2	Chronic pulmonary tuberculosis, far advanced	Whole serum	1:32	—		—	
		Supernatant A	1:16	Negative	1×	445	
		Precipitate B	1:8	1:4	1×	70	
		Precipitate C_1	1:4	1:16	1×	24	
3	Chronic pulmonary tuberculosis, far advanced	Whole serum	1:40	Negative		—	
		Supernatant A	1:2	Anticomplementary	1×	509	
		Precipitate B	Negative	Anticomplementary	1×	59	
		Precipitate C_1	1:2	Anticomplementary	1×	35	
4	Chronic pulmonary tuberculosis, moderately advanced	Whole serum	1:40	Negative		1,100	
		Supernatant A	Negative	—	3×	2,919	
		Precipitate B	Negative	—	3×	345	
		Precipitate C_1	1:256	—	5×	285	
5	Chronic pulmonary tuberculosis, far advanced	Whole serum	1:20	Negative		940	
		Supernatant A	1:8	Negative	2×	1,700	
		Precipitate B	1:8	Anticomplementary	2×	286	
		Precipitate C_2	1:16	1:8	2×	257	
6	Chronic pulmonary tuberculosis, far advanced	Whole serum	1:80	1:2		950	
		Supernatant A	1:64	Negative	2×	1,310	
		Precipitate B	Negative	1:2	2×	147	
		Precipitate C_1	1:128	1:8	2×	250	
7	Chronic pulmonary tuberculosis, far advanced	Whole serum	1:10	1:2		1,720	
		Supernatant A	Negative	Undiluted positive	2×	2,310	
		Precipitate B	Negative	1:8	2×	63	
		Precipitate C_1	1:8	1:8	2×	148	
8	Chronic pulmonary tuberculosis, moderately advanced	Whole serum	1:40	Undiluted positive		1,250	
		Supernatant A	1:8	Negative	2×	1,680	
		Precipitate B	1:128	1:2	2×	233	
		Precipitate C_1	1:4	1:16	2×	143	

TABLE 2

Serologic Results on Fractions of Tuberculous Sera Not Reacting in Hemagglutination Test

Serum Number	Diagnosis	Serum Fraction	Titer of Test		Concentration	Nitrogen in Mg. per 100 Ml.	Electrophoretic Pattern*
			Hemagglutination	Complement Fixation			
9	Chronic pulmonary tuberculosis, moderately advanced	Whole serum	Negative	Negative		999	
		Supernatant A	Negative	Negative	1×	640	
		Precipitate B	Negative	Negative	1×	78	
		Precipitate C_1	Negative	1:32	1×	22	
10	Chronic pulmonary tuberculosis, moderately advanced	Whole serum	Negative	Negative		1,200	
		Supernatant A	Negative	Negative	3×	2,181	
		Precipitate B	Negative	Negative	5×	260	
		Precipitate C_1	Negative	1:256	5×	235	
11	Chronic pulmonary tuberculosis, moderately advanced	Whole serum	Negative	>1:4		1,310	
		Supernatant A	Negative	1:2	3×	2,709	
		Precipitate B	Negative	1:512	5×	236	
		Precipitate C_1	Negative	±	5×	29	
12	Chronic pulmonary tuberculosis, far advanced	Whole serum	Negative	Negative		1,300	
		Supernatant A	Negative	Negative	2×	1,107	
		Precipitate B	Negative	1:2	2×	132	
		Precipitate C_1	Negative	1:16	2×	289	
13	Chronic pulmonary tuberculosis, minimal	Whole serum	Negative	Negative		1,220	
		Supernatant A	Negative	Negative	2×	1,720	
		Precipitate B	1:8	+	2×	207	
		Precipitate C_1	Negative	1:16	2×	102	
14	Chronic pulmonary tuberculosis, minimal	Whole serum	Negative	Negative		1,360	
		Supernatant A	Negative	Negative	1×	749	
		Precipitate B	Negative	Anticomplementary	2×	137	
		Precipitate C_1	1:8	1:8	2×	82	
15	Chronic pulmonary tuberculosis, far advanced	Whole serum	Negative	Negative		1,710	
		Supernatant A	1:64	Negative	2×	2,120	
		Precipitate B	Negative	Anticomplementary	2×	111	
		Precipitate C_1	Negative	1:8	2×	163	
16	Chronic pulmonary tuberculosis, far advanced	Whole serum	Negative	>1:16		1,300	
		Supernatant A	1:4	Negative	2×	1,830	
		Precipitate B	1:16	1:2	2×	256	
		Precipitate C_1	1:8	>1:16	2×	286	

TABLE 3

PROTEIN NITROGEN CONTENT OF NONTUBERCULOUS SERA AND FRACTIONS THEREOF

Serum Number	Diagnosis	PPD Skin Test	Serum Fraction	Concentration	Nitrogen in Mg. per 100 Ml.
17	Portal cirrhosis of liver	Positive	Whole serum		1,395
			Supernatant A	1×	1,140
			Precipitate B	2×	308
			Precipitate C_2	2×	143
18	Pneumonitis	Negative	Whole serum		1,340
			Supernatant A	1×	880
			Precipitate B	2×	384
			Precipitate C_2	2×	128
19	Myocardial infarct, carcinoma of right lung	Negative	Whole serum		1,530
			Supernatant A	1×	1,050
			Precipitate B	2×	257
			Precipitate C_2	2×	220
20	Obesity	Negative	Whole serum		1,325
			Supernatant A	1×	1,050
			Precipitate B	2×	290
			Precipitate C_2	2×	82
21	Chronic bronchitis, emphysema	Positive	Whole serum		925
			Supernatant A	1×	688
			Precipitate B	2×	127
			Precipitate C_2	2×	86

destroy antibody activity, the sera of 8 tuberculous patients (Nos. 9 to 16) which did not contain hemagglutinating antibodies were fractionated (table 2). Sera Nos. 11 and 16 gave a positive complement-fixation test. Fractions of only 4 of these sera (Nos. 13, 14, 15, and 16) reacted in the hemagglutination test. In the complement-fixation test, however, one or several of the fractions of all 8 sera gave positive results. In 7 instances the complement-fixing activity was mainly associated with fraction C_2; in the eighth (serum No. 11), with fraction B. It is of interest, therefore, to compare the electrophoretic pattern of fraction B of No. 11 with the patterns of No. 10, in which precipitate C_2 exclusively possessed antibodies. The latter consisted mostly of proteins of slow mobility comparable with that of gamma$_2$ globulin. Fractions B of Nos. 10 and 11 were similar in pattern as far as the slow-moving proteins are concerned. Fraction B of No. 10, however, which did not react, also contained a protein of the mobility of albumin. The possibility that the albumin fraction contained an inhibiting factor was suggested by this observation, and will be discussed below. It is further noteworthy that the fractions C_2 of sera Nos. 13 and 15 contained the complement-fixing antibody, while the hemagglutinating antibody was present in precipitate B of No. 13 and in supernatant A of No. 15.

For a control observation, the sera of 5 nontuberculous patients (table 3) were fractionated. Two of these patients were tuberculin reactors. Neither the whole sera nor their fractions, 11 of which were concentrated twice, revealed any antibody activity in either the hemagglutination or complement-fixation

TABLE 4

RESULTS OF COMPLEMENT FIXATION TEST ON COMBINED SERUM FRACTIONS

Experiment Number	Inactive Fraction			Active Fraction			Expected Result	Actual Result
	Serum Number	Fraction	Concentration	Serum Number	Fraction	Concentration		
a	9	A	3×	9	C_2	5×	1:16	1:8
b	9	B	5×	9	C_2	5×	1:16	Negative
c	Saline	—	—	9	C_2	5×	1:16	1:4
d	10	A	1×	10	C_2	5×	1:128	1:4
e	10	B	5×	10	C_2	5×	1:128	1:64
f	Saline	—	—	10	C_2	5×	1:128	1:64
g	12	A	2×	12	C_2	2×	1:8	1:8
h	16	A	2×	12	C_2	2×	1:8	1:8
i	12	A	2×	16	C_2	2×	1:8	1:16
j	16	A	2×	16	C_2	2×	1:8	1:8
k	13	A	2×	13	C_2	2×	1:8	±
m	8	A	2×	13	C_2	2×	1:8	1:2
n	13	A	2×	8	C_2	2×	1:8	1:8
o	8	A	2×	8	C_2	2×	1:8	1:8
p	5	A	2×	5	C_2	2×	1:4	1:4
r	14	A	1×	14	C_2	2×	1:4	1:2
s	15	A	2×	15	C_2	2×	1:4	1:2

test. An adequate amount of protein was present in these fractions, as may be seen by comparing the nitrogen values in table 3 with those in tables 1 and 2.

Combination of serum fractions: The results obtained with fractions demonstrated that antituberculous antibodies were present in the "false negative" sera, but could react only after fractionation. It was recognized that the incomplete separation of the protein fractions would not permit identification of the one with which the inhibiting factor is associated. It was hoped that, by combining various fractions, an indication might be obtained of whether an inhibiting substance was present at all. In these experiments (table 4) half of the amount of the active fraction used in the tests recorded in tables 1 and 2 was combined with an equal amount of an inactive solution. Since the latter was free of antibody activity, the 1:1 dilution should result only in a onefold reduction in titer, if a suppressing substance was not present. Reacting fractions derived from "false negative" sera were set up with nonreacting fractions of the same serum, and also with a corresponding fraction of a positive serum. Likewise, reacting fractions of positive sera were combined with fractions of "false negative" sera which hypothetically contained a suppressing factor.

It is considered significant that more than a twofold reduction in titer was observed when inactive fractions of sera Nos. 9, 10, and 13 were combined with their active fractions (*experiments b, d,* and *k,* table 4). When the suppressing fraction of *experiment k,* however, was combined with the active fraction of a positive serum (*experiment n*), no inhibiting effect was seen. Fractions of other "false negative" sera had no inhibiting effect upon the antibody-carrying protein of the same (*experiments g, j, r,* and *s*) or another serum (*experiments h* and *i*).

Likewise, inactive fractions of active sera did not inhibit their active counterpart (*experiments o* and *p*). The presence of an inhibiting substance, therefore, could not be deduced from the results of these experiments.

DISCUSSION

Inability to demonstrate circulating antibodies in a large number of patients with bacteriologic and clinical evidence of active tuberculous disease by so sensitive a procedure as the hemagglutination test requires investigation. The failure to manifest antibody activity may be the result of numerous factors, alone or combined. These fall into three groups: failure of the tissue to form or release antibodies; inhibiting factors inherent in the serum; and a rate of catabolism of antibodies more rapid than that of their formation.

The present investigations concern themselves with the first two of these possibilities. Plasma cells and lymphocytes have been suggested by several investigators as producers of circulating antibodies. Those sessile in lymph nodes are probably of importance in tuberculous infection, as demonstrated by Westwater (24), but the circulating leukocytes, as indicated by these experiments, could not be assumed to be involved in the problem under discussion. Felton (25) entertained the concept of immunologic paralysis. It seemed possible that the overwhelming amount of antigen which Felton employed in his experiments could be derived in tuberculous patients from the large number of tubercle bacilli ubiquitous in their lesions. If this were so, then in general, patients with far advanced extensive disease should be the poorest antibody formers. The contrary was found (7, 13), and this concept was not applied.

The concept that antibody activity associated with human serum protein may be inhibited by other proteins or substances present in the serum proved fruitful. Although separation of proteins by the method employed was incomplete, antibody activity could be demonstrated in one or several fractions of each serum which, as a whole, had failed to react. In 3 instances (Nos. 13, 14, and 15) both hemagglutinating and complement-fixing antibodies manifested themselves after fractionation; in 3 others (Nos. 9, 10, and 12), the latter type only manifested itself. Two sera containing complement-fixing (Nos. 11 and 16) and one containing hemagglutinating antibodies (No. 5) were revealed by fractionation to also harbor the other type. These observations suggested that an inhibiting substance was present in the sera. The results of attempts to localize this substance by combining various fractions were, however, inconclusive. Amino acids have been reported by Kleinschmidt (26) to inhibit precipitin reactions. If present, these should be contained in fraction A. The latter, indeed, in 2 instances seemed to affect inhibition. In others, however, it did not. Furthermore, the concentrations employed by Kleinschmidt were a multiple of those present in human sera. For these reasons, it is unlikely that amino acids are responsible for the suppression of antibody activity.

It is conceivable that the unmasking of antibody activity in these sera was due to dissociation of an antibody-antigen complex. This possibility is not excluded by the fact that reassociation did not occur when the positive and negative

fractions were combined, since the ethanol used in the procedure could have prevented reassociation (27). The problems of antigen-antibody complexes in sera of tuberculous patients and of unmasking antibody activity in other infectious diseases are being investigated.

The incomplete separation of proteins accomplished by the fractionation procedure did not permit exact localization of antibody activity. The complement-fixing antibodies, according to Baldwin and Iland (28), are contained in the gamma globulins, and so are the hemagglutinating antibodies (29). Some of the present results suggest that these two antibodies are associated with proteins of different physicochemical character. For instance, in cases Nos. 13 and 15, in which the hemagglutinating activity resided in fractions B or A, the complement-fixing antibody was associated with C_2. Separation of antibody molecules into fractions of different specificities by convection electrophoresis was observed by Cann and associates (16), and may have also occurred in the present experiments.

It is noteworthy that fractions of "false negative" sera revealed antibody activity with the complement-fixation procedure, but not with the more sensitive one employing Old Tuberculin-sensitized cells. Since the hemagglutinating type of antibody was not destroyed by the fractionation procedure in other tuberculous sera, the only explanation at this time would be that complement-fixing antibodies solely were present. That different types of antibody, the production of which was due probably to different constituents of the same causative agent, persist for different lengths of time has been reported, for instance, for coccidioidomycosis (10).

SUMMARY

Fractionation of serum proteins by the cold ethanol method unmasked antibody activity.

Of 8 specimens of tuberculous serum which did not react in the Middlebrook-Dubos test, 4 could be demonstrated by fractionation to contain hemagglutinating antibodies. The serum of 7 patients which failed to react in the complement-fixation test yielded protein fractions manifesting antibody activity.

The complement-fixing antibody was associated with the slow-moving globulins; the hemagglutinating antibodies could not be precisely located. Conclusive evidence for the presence of a substance suppressing antibody activity was not obtained.

Fractionation of sera from 5 nontuberculous patients, 2 of whom had positive tuberculin skin tests, did not yield fractions which reacted in the hemagglutination or complement-fixation test.

SUMARIO

Descubrimiento de Anticuerpos Circulantes Aparentemente Ausentes en Sueros Tuberculosos

La fraccionación de las seroproteínas por el método del etanol en frío reveló la actividad de los anticuerpos.

De 8 ejemplares de suero tuberculoso que no reaccionaban a la prueba de Middlebrook-Dubos, en 4 pudo descubrirse con la fraccionación que contenían anticuerpos hemaglutinan-

tes. Siete ejemplares de suero tuberculoso que no reaccionaron con la prueba de fijación del complemento rindieron fracciones de proteína que manifestaban anticuerpos activos.

Los anticuerpos que fijaban el complemento se asociaron con las globulinas de poca movilidad; no pudo localizarse con precisión a los hemaglutinantes. No pudieron obtenerse pruebas terminantes de la presencia de una substancia que suprima la actividad de los anticuerpos.

La fraccionación del suero de 3 sujetos no tuberculosos, 2 de los cuales acusaron cutirreacciones positivas a la tuberculina, no rindió fracciones que reaccionaran a las pruebas de la hemaglutinación o de la fijación del complemento.

RESUME

Détection dans des sérums tuberculeux d'anticorps circulants inapparents

Le fractionnement des protéines sériques par la «méthode de l'éthanol à froid» démasque l'activité anticorps.

Sur 8 échantillons de sérum tuberculeux ne réagissant pas au test de Middlebrook-Dubos, il a été possible de démontrer par le fractionnement la présence d'anticorps hémagglutinants dans 4 d'entre eux. Sept échantillons de sérum tuberculeux qui ne réagissaient pas au test de fixation du complément ont donné des fractions protéiques manifestant une activité anticorps.

L'anticorps fixant le complément était associé aux globulines lentes; les anticorps hémagglutinants n'ont pu être localisés avec précision. Les auteurs n'ont pas obtenu de preuves concluantes de la présence d'une substance réductrice de l'activité anticorps.

Le fractionnement du sérum de 5 malades non tuberculeux, dont 2 avaient une réaction positive à la tuberculine, n'a pas donné les fractions présentant une réaction positive à l'hémagglutination ou au test de fixation du complément.

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REPORT ON A FLOUROSCOPIC CHEST SURVEY IN CHINA (CANTON), JANUARY, 1948, TO FEBRUARY, 1949[1]

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(Received for publication June 4, 1954)

INTRODUCTION

The high incidence of tuberculosis morbidity and mortality in the city of Canton, China, had always been known to the local medical profession as well as to the laymen. One was always quite safe in venturing the remark that 20 per cent of the general practitioners' patients were suffering from tuberculosis, since no definite statistics were available. This was largely due to the lack of trained personnel and interest in tuberculosis work and to the lack of funds; and partly due to the lack of any definite active tuberculosis society within the city. The work of the present writers' clinic had always been in the field of diseases of the chest and particularly in tuberculosis. As it was known that the present group had conducted free tuberculin and fluoroscopic examinations of students in Hong Kong and Canton, various Canton private schools and organizations (banks, factories, teachers' associations, artists, the city police and jail, et cetera) approached the clinic to conduct similar surveys on their personnel. Accordingly, a fluoroscopic chest survey was conducted without the tuberculin tests. This was done because tuberculous infection was found to be universal in a previous study of a tuberculin and fluoroscopic survey on Chinese students in private schools in Hong Kong (2), where the student body was mainly Cantonese. Thus, due to the expected high incidence of positive tuberculin reactors, the tuberculin test was omitted and only fluoroscopy was used in a chest survey of 40,858 persons who were residents of Canton proper, from January, 1948, to February, 1949.

It is recognized that the only satisfactory and accurate way to conduct a case-finding survey is to obtain some permanent record, that is, miniature films which can be viewed and reviewed at leisure by more than one observer. It is further recognized that, if any suspicious or definite lesions are noted, large full-scale films should be taken and similarly reviewed in order to arrive at a conclusive diagnosis. Unfortunately, however, the facilities for obtaining roentgenograms on such a large scale were not available, as this survey was conducted by a privately financed clinic.[4] Nevertheless, it is believed that the results are sufficiently valuable from a purely statistical standpoint to justify the time, energy, and resources expended in a fluoroscopic survey of a large number of subjects and in checking the persons with any abnormal findings by means of large films. The survey, aside from its main purpose of case finding, presumably

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TABLE 1

FLUOROSCOPIC CHEST SURVEY IN CHINA (CANTON)

	Age in Years	Number Fluoroscoped			
		Male	Female	Total	Per Cent
Schools	11–20	19,467	7,948	27,415	67.10
Universities	21–25	6,622	839	7,461	18.26
Other	25 and older	5,090	892	5,982	14.64
Total		31,179	9,679	40,858	100.00

served some purpose in tuberculosis education and in arousing public interest in tuberculosis, and made some contributions to its present enthusiasm and high spirit in the support of a good sound tuberculosis program. Finally, the writers were pleased to have the interest and hearty cooperation of the local medical practitioners and public health department as the work progressed.

GENERAL PLAN OF SURVEY

Subject Matter

The survey was not confined to any specific district of the city, but was scattered throughout, e. g., in the slums of the thickly populated sections as well as in the less dense areas. No attempt was made to regulate or choose the type of schools and organizations. The rich and the poor were examined alike without distinction, since the work was done on voluntary requests and invitations to the Chest Clinic. No invitations were ever refused because of small numbers or distance. Several organizations had only 40 to 50 members and were sometimes a distance of six to ten miles from the clinic over rough roads. Thus, the survey was in no way a cross section of the general population as a whole.

The schools in which the survey was conducted were for the most part private in character in that students were required to pay a tuition fee. Fifty-one schools accepted the survey, in which 27,415 students were fluoroscoped. Forty-eight were middle schools and three were primary. The eight universities (although in most cases large fees were not collected) were attended by persons of the upper and middle classes, whose standard of living was considerably higher than that of the average Chinese family. As may be seen in tables 1, 2, and 3, more than 67 per cent of the total group examined was made up of elementary and middle school children, 18 per cent university students, while approximately 15 per cent of the other groups were more than twenty-five years of age.[5]

The other organizations were made up of students of thirteen special schools and employees and inmates of eighteen concerns in the city. These are tabulated in table 3. The prisoners in the city jail, contrary to expectation, were found to

[5] It should be noted in this regard that, since a Chinese baby is considered one year old at birth, and as its age advances each Chinese New Year, the ages listed in the following report vary from one to as much as two years more than they would according to Western standards.

TABLE 2

FLUOROSCOPIC CHEST SURVEY IN CHINA (CANTON)

Age in Years	Number			Cases of Pulmonary Tuberculosis			
	Male	Female	Total	Roentgenography	Fluoroscopy*	Total	Per Cent
6–10				6		6	
11–15	6,698	2,910	9,608	89	2	91	0.95
16–20	12,769	5,038	17,807	265	47	312	1.75
21–25	6,622	839	7,461	186	64	250	3.35
26–30	2,251	380	2,631	102	21	123	4.67
31–35	1,225	224	1,449	84	20	104	7.18
36–40	844	146	990	57	18	75	7.57
41–45	363	67	430	30	16	46	10.70
46–50	213	41	254	19	10	29	11.42
51–75	194	34	228	17	18	35	15.39
Total	31,179	9,679	40,858	855	216	1,071	2.62

* See table 4 and *Observations* in the text.

be quite well nourished and fairly healthy, probably due to the fruits of their previous occupations. However, the former groups were for the most part in a low income bracket, with barely enough food to subsist and living in very crowded and unsanitary quarters.

Method of Conducting Survey and Plan of Investigation

The project in a sense represented a traveling survey in that each institution was visited in turn and a darkroom had to be set up prior to the arrival of the survey team. Often great difficulty was experienced in assembling the darkroom and obtaining the proper electric current, but it was nevertheless found feasible to complete the examination in practically all cases.

The machine used was a small portable 15MA X-ray (oil-immersed tube head) with a strength current of 90 kilovolts; 2.5 milliamperes were used, and the tube screen distance was 36 inches. A 14-by-16-inch Patterson fluoroscopic screen, mounted on a wooden frame with lead glass of the same size, was used and placed over a makeshift wooden stand. A 16-by 24-inch lead rubber attached to the bottom of the screen frame and hanging downward protected the operator from direct and secondary radiation. The framed screen with the lead rubber could be raised and lowered, since a stem was attached to it, the other end of which passed through a hole in the center of the wooden stand. The tube head focus was leveled with the rays directed to and covering the whole of the screen, and a finer focus adjustment with a long lever (36-inch) attachment to shutters in front of the tube extended to within easy reach of the operator. This enabled the latter to center more directly on any part of the chest. The height of the screen was more or less fixed to include the entire chest heights of the subjects. The subjects were arranged in groups of approximately the same heights, and flat stools of 4 inches and 12 inches were used for shorter subjects to stand on, as the case might require. A step-up transformer with voltage steps from 50 volts to 250 volts was always on hand to step up the line voltage to the constant 210 to 230 volts required by the X-ray machine. This was an absolute necessity, as the Canton city electricity varied from 90 to 200 volts in different districts. An ice bag with constant change of fresh ice was kept on top of the tube head to keep the temperature down and thus prevent overheating the tube. A 16-inch desk electric fan was used to cool the darkroom when

TABLE 3

FLUOROSCOPIC CHEST SURVEY IN CHINA (CANTON)

Schools and Organizations	Number
Colleges (universities)	8
Middle schools	48
Primary schools	3
Special schools	13
Provincial Industrial School	
Provincial Physics	
Law and Commerce	
Literature and Science	
No. 2 Overseas Teachers' Institute	
Women's Provincial Teachers' Institute	
No. 1 Training Institute (occupational therapy for prisoners)	
No. 2 Training Institute	
Midwife School	
Art and Music	
Literature and Law	
School of Agriculture	
Municipal Teachers' Institute	
Miscellaneous	18
Supreme Court	
Provincial Police Head Quarters	
Canton Jail prisoners	
Canton Ice Manufactory	
Institute for the Blind	
Water Works Office	
Teaching Department	
Education Department	
Central News Supply	
Salt Bureau	
Y.W.C.A.	
Post Office	
Marine Office	
Telegraph Office	
Bank of China	
Nutrition Center	
Staff of Jail	
City Police Station	

necessary. The fan, although revolving from side to side, was usually placed on the floor five feet away to the side and behind the operator, who did most of the work. Other incidentals were some electrical wirings, plugs, adhesive tapes, a small screwdriver, a pair of pliers, and a small hammer with a nail remover to help arrange the survey in working order. A large 1942 chauffeur-driven six-cylinder Plymouth passenger car with a baggage compartment solved the transportation problem. Two physicians, two helpers, and all of the above equipment were thus transported to the various places of the survey.

The day of, or the morning before, the fluoroscopic survey was conducted in a definite school or organization, a physician and one or two nonprofessional personnel were sent out to look over the place. The physician would discuss with the responsible heads the details and arrangements for the conduct of the survey. A suitable room would be selected and prepared with the proper electrical connections and, on the day of the survey, heavy blue

cloths were used to cover the windows, transoms, and other possible leakage of external light, thus making the room dark.

Personnel required were one physician and an operator for the machine, using the interrupted hand switch, besides several checkers who were recruited from the institution concerned for recording purposes. Two or more responsible staff members received specific instructions to arrange for an orderly line-up of 30 subjects waiting continuously outside of the darkroom. Each one of the subjects held a card with the name, age, sex, grade, position held (if a teacher), and number of classrooms written on it. Each card was collected individually while the subject was being fluoroscoped, and the examining physician put aside the ones with suspicious or abnormal findings after making some notes on them, if necessary. These were then referred for further examination, and roentgenograms were obtained. All of the cards collected were checked against a list of the members in each class in order to find out the number of subjects who did not turn up for examination and, if possible, the reasons why.

In most cases the examination was compulsory, so that almost 100 per cent attendance was obtained, thus making the survey as representative as possible. Among the older age groups, in which compulsion was impossible, it is believed that many were not examined in the groups because they thought that they might have some lung disease and preferred to remain in doubt on the question. Undoubtedly, others came for examination especially because they believed they were suffering from tuberculosis. Considering all of the factors, it was estimated that these two discrepancies probably balanced one another.

The subjects were fluoroscoped in groups of 10 or 15, depending upon the size of the darkroom and, with good organization, it was found possible to complete approximately 60 to 80 examinations in one hour. When any shadow was seen in the lung parenchyma which was not obviously small and calcified, the subject was required to report as soon as possible to have a large chest postero-anterior roentgenogram taken. A total of 1,339 persons were found to have suspicious lesions on fluoroscopy; of these, 1,069 had follow-up chest films taken (tables 1 and 2). Following the reading of the films, a report was sent to the individual concerned through the responsible school authorities. If there was significant disease, tuberculosis or otherwise, the person was advised to report to his family physician; if the disease was obviously healed or the film was normal, he was advised to that effect. Strict medical ethics were observed in that identification of the survey team with the private clinic was not disclosed. Indeed, since no fees were imposed, most of the people thought that the team was sent by the city health authorities. The presence of a Canadian physician[6] made it all the more impressive in this respect.

Abnormalities in the pleura without changes in the lung parenchyma were not recorded, nor were some of the bony abnormalities of the ribs or thoracic spine. Heart shadows which were obviously enlarged, as well as marked changes in the aortic shadows, were noted and particular attention was paid to dextrocardia. In order to clarify the results, establish the usage of some of the terms, and give an accurate picture of certain variables and possible inaccuracies, some explanations are in order.

Tuberculosis: In the category *tuberculosis* were included any form of reinfection pulmonary tuberculosis as well as changes characteristic of the primary type, whether apparently healed or progressive, and extensive changes in the pleura. Small healed primary complexes or minimal pleural changes were not included.

Normal: Those films without evidence of significant tuberculosis or other pulmonary disease were called *normal*.

[6] Dr. R. M. Chadwick.

Miscellaneous conditions: Pulmonary infiltration or changes that did not have the appearance of tuberculous etiology were included in the category *miscellaneous.* Some of these, particularly those showing pneumonic involvement of the mid and lower portions of the lung fields, may and undoubtedly were due to tuberculosis because other criteria necessary for an accurate diagnosis were not available. This factor would reduce the over-all percentage of significant disease found by a very small amount.

Overlooked cases: Undoubtedly, a small number of cases of minimal pulmonary tuberculosis were overlooked during the examination. The number would depend upon the experience and accuracy of the examiner. As several very early small lesions were discovered, however, it was believed that the number of cases overlooked was not great. Whenever any doubt was present, a roentgenogram was always obtained in an obvious attempt to "overdiagnose" on the fluoroscopic examination.

Unsatisfactory films: Approximately 1 per cent of the films was technically unsatisfactory, in that no definite decision could be reached whether tuberculosis was present. This group is listed as *unsatisfactory films.*

OBSERVATIONS

As may be seen in tables 1 and 2, by far the greater percentage of subjects were in the eleven- to twenty-five-year age group, and the resultant percentages are probably accurate for these groups. Correspondingly, the greatest number of tuberculosis cases was found in these groups, while the percentage was lower in comparison with the older age groups.

Roentgenograms were obtained for 1,069 subjects who were diagnosed by fluoroscopic examination as having lesions suspicious of tuberculosis. Of these, 855 (approximately 80 per cent) were considered to have pulmonary tuberculosis; 111 (10.38 per cent) were designated as normal; 89 (8.33 per cent) were found to have pulmonary disease not considered tuberculous in etiology; and 14 films (1.31 per cent) were considered unsatisfactory.

A group of 270 persons, although considered to have pulmonary abnormality on fluoroscopic examination, were not examined roentgenographically. Of these, 163 did not come for roentgenographic examination, while 107 prisoners could not come. The number of more than 1,000 persons who were examined roentgenographically was moderately large and representative. Accordingly, it was believed that, if the group who did not have roentgenograms were subdivided

TABLE 4

FLUOROSCOPIC CHEST SURVEY IN CHINA (CANTON)

Roentgenograms Taken	Number	Percentage
With significant tuberculosis	855	79.98
Normal (no lesions found)	111	10.38
Conditions other than tuberculosis (including heart, bone, et cetera)	89	8.33
Unsatisfactory films	14	1.31
Total	1,069	100.00

according to the results found in the broad divisions of those films which were taken, too great an inaccuracy would not exist. It was assumed, therefore, that 216 of this group of 270 would have significant disease, 27 would have normal films, et cetera (table 4). Actually this reasoning, although statistically inaccurate, is probably on the conservative side, since in the group of prisoners (107 subjects) more than 90 per cent were considered to have moderately to far advanced lesions by fluoroscopy.

If these 270 had been examined roentgenographically and if the results were similar to those on whom roentgenograms were actually obtained, assuming the amount of tuberculosis found would be comparable, they could be subdivided as follows:

	Per Cent	Number
With significant tuberculosis	79.98	216
Normal (no lesions found)	10.38	28
Conditions other than tuberculosis	8.33	23
Unsatisfactory films	1.31	3
Total	100.00	270

Of the 855 persons who had films taken and were considered to have significant pulmonary tuberculosis, 714 had lesions which were classified as reinfection type; while, in 141, the lesions were designated as primary type, pulmonary, hilar, or mixed. There were 382 minimal, 298 moderately advanced, and 34 far advanced cases of pulmonary tuberculosis as shown in table 5. The value for active pulmonary tuberculous activity (565 cases) was achieved by using no criteria other than the roentgenographic appearance, since it was not possible to make sputum examinations or follow-up films.

It is of interest to consider the subjects who were found to have pulmonary infiltration not considered tuberculous in origin, including those who, although having obvious tuberculous disease, also presented other lesions not considered tuberculous. These data are presented in table 6. Infiltrations in the lower portions of the lung fields usually had the appearance of chronic, nonspecific, inflammatory lesions, perhaps due to bronchiectasis; while the apparently non-

TABLE 5

Fluoroscopic Chest Survey in China (Canton)

Classification of Significant Pulmonary Tuberculosis Found

	Side				Pleural Involvement	Cavitation Honeycombing	Active
	Right	Left	Bilateral	Total			
Primary	54	34	53	141	45		47
Minimal	122	91	169	382	36		196
Moderately advanced	59	40	199	298	35	208	289
Far advanced	2	1	31	34	7	31	33
Total	237	166	452	855	123	239	565

TABLE 6

FLUOROSCOPIC CHEST SURVEY IN CHINA (CANTON)

		Non-tuberculous	Tuberculous
Infiltration	Right upper	9	
	Right middle	14	2
	Right lower	15	6
	Left upper	2	
	Left middle	7	
	Left lower	13	2
		19	2
Including bronchial vascular shadows		8	
Fibrosis			
Pneumothorax	Right		3
	Left		5
	Bilateral		1
	Spontaneous		1
Oleothorax			1
Foreign body (bullet)			1
Miliary tuberculosis			1

tuberculous infiltrations in the upper portions were more pneumonic in character. Diffuse pulmonary fibrosis was present in 8 persons. Several patients receiving collapse treatment for tuberculosis were detected, as well as one case of oleothorax (from the history). One foreign body, a bullet, was also seen.

Many changes in the bony thorax, both congenital and acquired, were noted both on fluoroscopic examination and on some of the roentgenograms which were taken but, due to the press of work, it was impossible to record them all. The impression was reached, however, that cervical rib was not as common as is usually found in the United States, but underdeveloped ribs and synostoses were quite numerous (table 7).

Cardiac abnormalities were not very common, probably due to the fact that

TABLE 7

FLUOROSCOPIC CHEST SURVEY IN CHINA (CANTON)

Bony Abnormalities

	Roentgenogram	Fluoroscopy	Total
Small first rib	6	2	8
Small second rib	1	0	1
Cervical rib	2	2	4
Synostosis	4	4	8
Potts' disease	2	0	2
Scoliosis	7	2	9
Rib resection*	1	0	1
Sprengel's deformity with multiple osteomata	1	0	1
Total	24	10	34

* Part of one rib removed for drainage of empyema.

TABLE 8

FLUOROSCOPIC CHEST SURVEY IN CHINA (CANTON)

	Fluoroscopy	Roentgenograms		Total
		No tuberculosis	With tuberculosis	
Enlarged heart	42	4	6	52
Aortic arch enlarged	12	1	0	13
Dextrocardia	7	0	0	7
Total	61	5	6	72

the groups examined were for the most part healthy and also because of the preponderantly young age group (table 8). Nevertheless, there were 52 patients with grossly enlarged heart and 13 obviously enlarged aortic shadows. Many of the subjects with abnormal heart and large vessel shadows were examined clinically, and those more than thirty years old for the most part were suffering from syphilis; while from age twenty to thirty, mitral stenosis was the common lesion. In many subjects, no obvious lesion was apparent and they might have been suffering from vitamin deficiencies; some of the younger ones undoubtedly had congenital lesions.

Of more interest than the above, particularly since the value should be absolutely accurate, there were 7 cases of dextrocardia. The criteria for diagnosing this abnormality were heart shadow on the right, a right-sided aortic knob, and stomach bubble on the right, so that these 7 cases really represented transposition of the viscera as well. This would make the incidence of dextrocardia with transposition of viscera approximately one in 5,800 examined. There were no obvious cases of dextrocardia without transposition of the viscera.

DISCUSSION

In the last decade, roentgenographic surveys of the general population and special groups have been conducted in various tuberculosis centers throughout the world. Many new cases of tuberculosis have been discovered through this method of case finding. The high cost in carrying out the surveys, together with the fewer significant cases found, has caused comment in some communities on the practical application of the roentgenogram as so extensively used. Some workers advocate that tuberculin surveys (7) should be more extensively used; and photofluorography, with certain necessary reservations. It is obvious in certain parts of the world that tuberculin is the method of choice in the younger age group and is much more economical. However, the high incidence of tuberculosis found in China (1, 2, 6) justifies the continued use of the roentgenogram in mass examination for tuberculosis in the future.

The incidence of tuberculosis gradually increases in the older age groups (*see* table 2). Thus, an incidence of only 0.95 per cent in the eleven- to fifteen-year age group rose to 15.3 per cent in the fifty-one- to seventy-five-year age group. This finding was in agreement with the findings elsewhere in China (6).

The present survey cannot be considered as a cross section of the general population of Canton as a whole. The schools and universities were private in nature, inasmuch as tuition was required of all who attended, and the students were from families in relatively good economic circumstances. There were approximately 100,000 or more poor students attending public schools in the city who were not examined; and another 300,000 did not have the means to attend any type of school. Finally, the number of persons attending the special schools and the miscellaneous groups which comprised only 14.64 per cent (5,982) of the subjects surveyed (*see* table 1) was much too small to be conclusive when one takes into consideration that the population of Canton was approximately two and one-half million. Moreover, a great majority of the people were living in unsanitary and congested areas. It would not be surprising, therefore, to find a much higher tuberculosis morbidity in the latter groups than that shown in the present survey.

SUMMARY

A fluoroscopic chest survey in Canton, China, from January, 1948, to February, 1949, was made on 40,858 students of seventy-two private and special schools and on the staffs of eighteen organizations (banks, marine office, factories, police department, telegraph office, water works department, convicts, institute for blind, et cetera). A total of 1,071 cases of pulmonary tuberculosis were found: 855 confirmed by roentgenographic examination and 216 without. Final analysis of the 855 cases showed 565 to be active tuberculosis, of which 47 cases were primary, 196 minimal, 289 moderately advanced, and 33 far advanced. Nonspecific inflammatory lesions with and without tuberculosis were noted; many changes in the bony thorax were seen; and 7 cases of dextrocardia with transposition of viscera were also found.

SUMARIO

Informe Relativo a una Encuesta Roentgenoscópica del Tórax en China (Canton), Enero, 1948–Febrero, 1949

En Cantón, China, ejecutóse, de enero de 1948 a febrero de 1949, una encuesta fluoroscópica del tórax en 40,858 alumnos de setenta y dos escuelas particulares y especializadas y en el personal de dieciocho establecimientos (bancos, oficina marítima, fábricas, departamento de policía, oficina telegráfica, departamento de abastecimiento de agua, presidiarios, instituto para ciegos, etc.). Descubrióse un total de 1,071 casos de tuberculosis pulmonar: 855 confirmados por el examen radiográfico y 216 sin confirmación. El examen final de los 855 casos reveló que 565 eran activos, siendo 47 de ellos primarios, 196 mínimos, 289 moderadamente avanzados y 33 muy avanzados. Notáronse lesiones inflamatorias anespecíficas con y sin tuberculosis; se observaron muchas alteraciones en la porción ósea del tórax; y además se encontraron 7 casos de dextrocardia con transposición de vísceras.

RESUME

Rapport sur une investigation radioscopique thoracique en Chine (Canton), janvier 1948–février 1949

Un programme de dépistage de la tuberculose par radioscopie thoracique a été effectué à Canton (Chine) de janvier 1948 à février 1949. Il comprenait 40 858 sujets, des étudiantes

de soixante-douze écoles privées et spécialisées et le personnel de dix-huit organismes (banques, bureaux de la marine, usines, police, Administration du télégraphe, Service des eaux, prisonniers, Institut des aveugles, etc). Au total, 1.071 cas de tuberculose pulmonaire ont été découverts; 855 ont été confirmés par la radiographie et 216 sans radiographie. L'analyse finale des 855 cas a montré 565 cas de tuberculose active dont 47 primo-infection, 196 cas a minima, 289 au stade modérément avancé et 33 cas très avancés. Des lésions inflammatoires non-spécifiques, associées au non à la tuberculose, ont été enregistrées ainsi que de nombreuses altérations osseuses du thorax; on a également découvert sept cas de dextrocardie avec transposition des viscères.

Acknowledgments

The writers are very much indebted to the physicians and nursing staff of the Canton Co-Op Chest Clinic, to the Canton Health Department, the staff of the schools and various organizations for their patient courtesies and hearty cooperation in making the survey pleasantly possible.

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ANTITUBERCULOUS ACTIVITY OF TETRACYCLINE AND RELATED COMPOUNDS[1]

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(Received for publication May 27, 1955)

INTRODUCTION

The antituberculous activity of oxytetracycline was originally described in a brief communication from this laboratory (1). Other investigators have subsequently reported that, although considerably less active than streptomycin, this antimicrobial agent does possess a degree of antituberculous activity which is demonstrable both in experimental animals and in man (2, 3). It has been reported that the degree of activity is adequate to delay the emergence of streptomycin-resistant strains in patients who receive these two drugs concomitantly (4, 5). The introduction of tetracycline in 1953 and the close relationship between this compound and oxytetracycline aroused interest in the possibility that it might possess similar antituberculous activity.

The present study was undertaken, therefore, to compare the respective antituberculous activities of tetracycline, oxytetracycline, and chlortetracycline *in vitro* and in experimentally infected mice.

MATERIALS AND METHODS

Crystalline tetracycline hydrochloride, crystalline oxytetracycline hydrochloride, and crystalline chlortetracycline hydrochloride were used throughout the study. In some instances, streptomycin sulfate, viomycin sulfate, and isoniazid were included for comparison. *In vitro* susceptibility tests were performed in the conventional manner, using a liquid Tween®-albumin medium and an incubation period of 12 to 14 days (37°C.). *In vivo* studies were conducted in male white mice (Millerton or Rockland Farm Swiss strain) infected either intravenously with the Vallée bovine strain or intracerebrally with the H37Rv human strain of *Mycobacterium tuberculosis*. Animals infected with the Vallée strain were treated in the conventional manner, by the oral route, once daily, from the day of infection until the time of death or sacrifice. Animals infected with the H37Rv strain, on the other hand, were treated by the subcutaneous route once daily for a period of 31 days, beginning on the day of infection. The effect of each antimicrobial agent upon the course of the infection was measured in terms of the average survival time and in terms also of the percentage of animals surviving at arbitrarily selected observation periods, i.e., at 28, 35, 42, 49, 56, and 63 days after infection with the Vallée strain, and at 30, 60, 90, and 180 days after infection with the H37Rv strain.

EXPERIMENTAL OBSERVATIONS

The *in vitro* susceptibility of thirteen strains of *M. tuberculosis* to tetracycline, oxytetracycline, and chlortetracycline, when tested simultaneously in a liquid Tween®-albumin medium at pH 6.8, is illustrated in table 1. In like manner, in table 1 is shown the *in vitro* susceptibility of these same strains to streptomycin, viomycin, and isoniazid when tested simultaneously with tetracycline, oxytetracycline, and chlortetracycline in the same medium and at the same pH.

[1] From the Research Division of Chas. Pfizer & Co., Inc., Brooklyn, New York.

TABLE 1

THE IN VITRO TUBERCULOSTATIC ACTIVITY OF OXYTETRACYCLINE, TETRACYCLINE AND CHLORTETRACYCLINE, AS COMPARED WITH STREPTOMYCIN, VIOMYCIN, AND ISONIAZID

(pH 6.8)

Strain of *M. Tuberculosis**	Susceptibility: γ per Ml. (12 days, 37°C.)					
	Tetracycline	Oxytetracycline	Chlortetracycline	Streptomycin	Isoniazid	Viomycin
H37Rv	1.6	31.0	31.0	0.31	0.16	2.50
H37Rv (INH-R)	6.3	31.0	31.0	0.31	4.00	2.50
H37Rv (PAS-R)	0.8	16.0	31.0	0.16	0.16	2.50
H37Rv (NM-R)	1.6	31.0	31.0	0.31	0.16	10.00
H37Rv (SM-R)	6.3	31.0	31.0	>1,000.00	0.16	2.50
H37Rv (*1075-ST)	3.1	31.0	31.0	>1,000.00	0.08	2.50
Vallée	1.6	16.0	16.0	0.16	0.31	1.25
Vallée (INH-R)	0.4	8.0	16.0	0.63	63.00	2.50
BCG (Phipps)	0.8	2.0	2.0	0.04	0.04	0.63
Wild strains						
Cummins	6.3	63.0	63.0	0.31	0.16	2.50
Marshall	1.6	16.0	31.0	1.25	0.16	2.50
Munkert (VM-R)	1.6	31.0	16.0	0.63	—	31.00
McVay (VM-R)	<0.2	<2.0	<2.0	<0.04	<0.02	31.00

* INH-R, PAS-R, NM-R, SM-R, and VM-R designate that these are mutant strains made resistant in the laboratory to isoniazid, para-aminosalicylic acid, neomycin, streptomycin, and viomycin, respectively. *1075-ST indicates resistance to both streptomycin and streptothricin.

TABLE 2

THE EFFECT OF PH ON THE IN VITRO TUBERCULOSTATIC ACTION OF TETRACYCLINE, OXYTETRACYCLINE, CHLORTETRACYCLINE, STREPTOMYCIN, VIOMYCIN, AND ISONIAZID

Strain of *M. Tuberculosis**	pH of Medium	Susceptibility: γ per Ml. (12 days, 37°C.)					
		Tetracycline	Oxytetracycline	Chlortetracycline	Streptomycin	Isoniazid	Viomycin
H37Rv	6.8	3.1	25.0	12.5	1.6	0.4	3.1
	5.5	0.8	3.1	3.1	6.3	0.4	12.5
H37Rv (*1075-ST)	6.8	3.1	12.5	25.0	>100.0	0.4	3.1
	5.5	1.6	3.1	0.8	>100.0	0.4	3.1
H37Rv (2-INH-R)	6.8	6.3	100.0	50.0	0.8	63.0	1.6
	5.5	3.1	25.0	1.6	3.1	63.0	50.0
H37Rv (PAS-R)	6.8	6.3	6.3	12.5			
	5.5	0.8	1.6	0.8			
Vallée	6.8	1.6	25.0	6.3			
	5.5	1.6	3.1	1.6			
Wild strains							
Munkert (VM-R)	6.8				0.8	1.6	12.5
	5.5				0.8	1.6	12.5
Cummins	6.8				0.4	0.8	3.1
	5.5				0.8	0.4	3.1
Moschen	6.8				0.8	1.6	3.1
	5.5				0.8	0.8	3.1
Beluk	6.8				1.6	0.8	1.6
	5.5				1.6	0.8	6.3

* *See* footnote to table 1.

It is apparent from these data that tetracycline is considerably more active *in vitro* than oxytetracycline or chlortetracycline. It is also apparent that chlortetracycline, like oxytetracycline, does possess some antituberculous activity. The *in vitro* activity of tetracycline against tubercle bacilli, when tested under the conditions of these experiments, is approximately as great as that of viomycin.

The possibility that the tuberculostatic action of certain antimicrobial agents may be influenced by the pH of the environment has been suggested in a recent report by McDermott and Tompsett (5). As shown in table 2, the activity of tetracycline, oxytetracycline, and chlortetracycline is enhanced at pH 5.5, while that of streptomycin and viomycin is frequently diminished in the acid range. As previously reported by McDermott and Tompsett, the activity of isoniazid appears to be unaltered by such change in the pH of the environment.

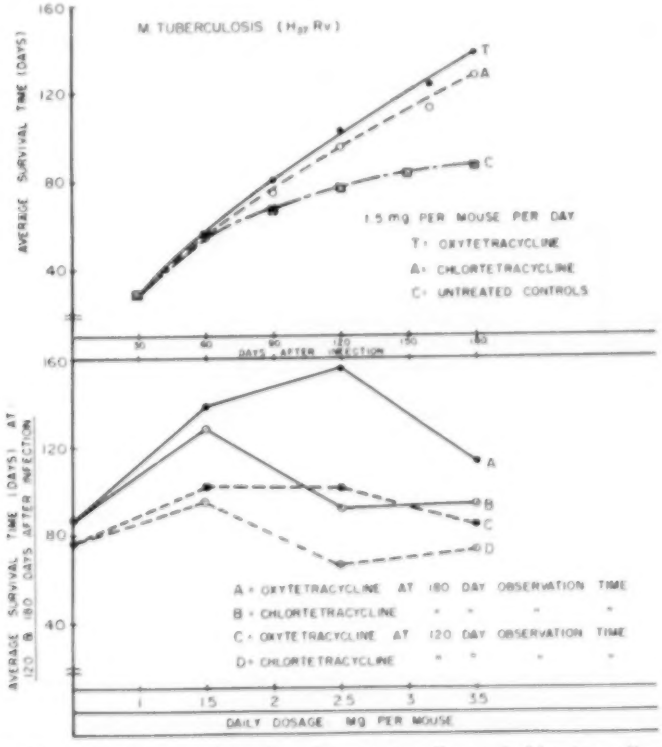

FIG. 1. The chemotherapeutic action of oxytetracycline and chloretracycline against tuberculous infections in mice produced by the intracerebral inoculation of *M. tuberculosis*, strain H37Rv. In all instances, treatment was administered subcutaneously once daily (five days weekly) for a period of 31 days after infection. The average results obtained in four separate experiments are shown.

It is of special interest that, in the acid range, the activities of tetracycline and of streptomycin are approximately of the same magnitude.

The *in vivo* activities of tetracycline and related compounds are illustrated in figures 1 and 2. From the data presented, it is apparent that tetracycline, as well as oxytetracycline and chlortetracycline, is active *in vivo* against *M. tuberculosis*. In the experiments reported herein, however, tetracycline was only as active as oxytetracycline *in vivo*, despite its apparently greater degree of activity *in vitro*.

Chlortetracycline and oxytetracycline, when used in low dosage, were equally active in the control of experimental tuberculous infections in mice. When high

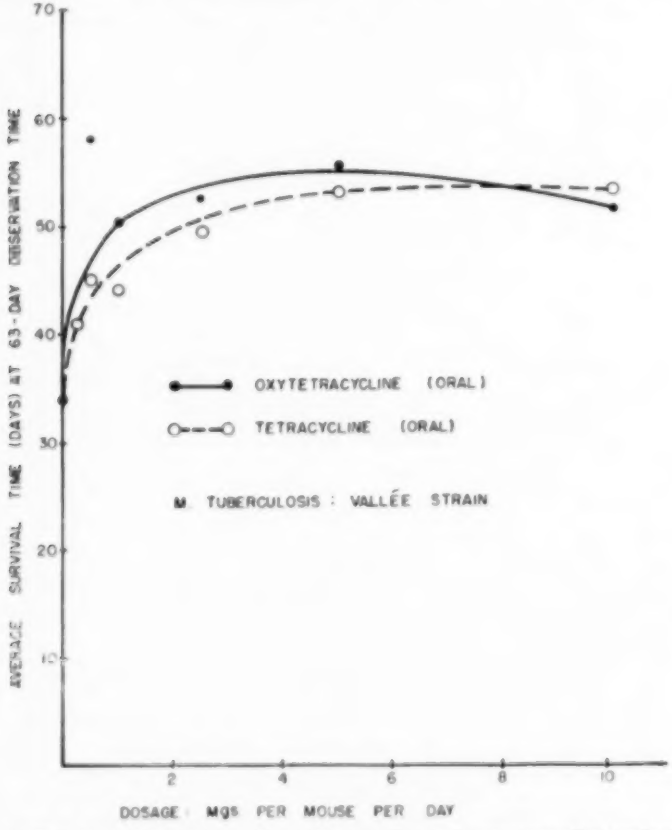

FIG. 2. The chemotherapeutic action of tetracycline and oxytetracycline against tuberculous infections in mice produced by the intravenous inoculation of *M. tuberculosis*, Vallée strain. In all instances, treatment was administered once daily (five days weekly) by the oral route from the day of infection until the day of death or sacrifice. The average results obtained in two separate representative experiments are shown.

dosages were used, both compounds were less uniform in action and indeed appeared to be relatively ineffective. As stated previously, the effect of these compounds was measured only in terms of the average survival time of infected animals, and no information is available concerning the extent of disease as evidenced by histopathologic observations. The shorter average survival time noted at 120, 150, or 180 days after infection, in animals treated with 2.5 to 3.5 mg. of oxytetracycline or chlortetracycline daily, as compared with those treated with only 1.5 mg. of each drug daily, cannot be explained on the basis of cumulative drug toxicity. The difference became apparent only after an interval of 90 to 120 days following cessation of therapy, i.e., at 120 to 150 days in animals treated for only 31 days after infection. One would anticipate, moreover, a lower relapse rate among animals treated with the larger doses, and the phenomenon noted herein remains unexplained.

DISCUSSION

From the limited amount of experimental data presented herein, it is apparent that tetracycline is at least as effective against *Mycobacterium tuberculosis*, both *in vitro* and *in vivo*, as oxytetracycline and chlortetracycline. The fact that the antituberculous action of tetracycline *in vitro* is enhanced in the acid range of pH suggests the possibility that at times it may be more effective against organisms existing within areas of inflammation or necrotic tissue. Indeed, based on the data presented, it is conceivable that tetracycline may be as effective as streptomycin when acting within such an environment. Although tetracycline was no more active than oxytetracycline and chlortetracycline in the *in vivo* studies described, this may well be due to the acute nature of the disease produced in mice by the intravenous inoculation of the strain used, i.e., the Vallée (bovine) strain of *Mycobacterium tuberculosis*. No significant difference was noted between the degree of antituberculous activity exerted *in vivo* by oxytetracycline and by chlortetracycline under the experimental conditions used.

SUMMARY

Tetracycline, oxytetracycline, and chlortetracycline are capable of inhibiting the growth of *Mycobacterium tuberculosis* both *in vitro* and *in vivo*. The *in vitro* activity of these compounds, in contrast to that of streptomycin or viomycin, is not diminished and at times may be enhanced in an acid environment. Although tetracycline appears to be more active *in vitro* than the other two drugs, chemotherapeutic studies in experimentally infected tuberculous mice failed to reveal any difference among the three compounds.

SUMARIO

Actividad Antituberculosa de la Tetraciclina, la Oxitetraciclina, y la Clortetraciclina

La tetraciclina, la oxitetraciclina y la clortetraciclina son capaces de inhibir, tanto *in vitro* como *in vivo*, la proliferación del *M. tuberculosis*. La actividad *in vitro* de estos compuestos, en contraposición a la de la estreptomicina o la viomicina, no disminuye, y a veces quizás hasta aumente, en un ambiente ácido. Aunque la tetraciclina parece ser más activa *in vitro* que las otras dos drogas, los estudios quimioterapéuticos ejecutados en ratones

tuberculosos infectados experimentalmente no revelaron la menor diferencia entre los tres compuestos.

RESUME

Activité antituberculeuse de la Tétracycline, l'Oxytétracycline, et la Chlortétracycline

La tétracycline, l'oxytétracycline et la chlortétracycline ont la capacité d'inhiber le développement du *Mycobacterium tuberculosis in vitro* et *in vivo*. L'activité de ces composés *in vitro*, en contraste avec celle de la streptomycine ou de la viomycine, n'est pas diminuée et peut même parfois s'accroître en milieu acide. Bien que, *in vitro*, la tétracycline apparaisse plus active que les deux autres composés, les études chimiothérapeutiques effectuées chez des souris infectées expérimentalement n'ont révélé aucune différence entre les trois composés.

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Case Reports

PNEUMATOSIS CYSTOIDES INTESTINALIS[1]

An Incidental Finding During Therapeutic Pneumoperitoneum

BERNARD LOWENSTEIN, JOHN W. BERRY, AND JAMES H. CUYKENDALL

(Received for publication March 21, 1955)

INTRODUCTION

Pneumatosis cystoides intestinalis (gas cysts of the intestine) is a rare condition characterized by multiple grapelike gas-containing cysts in some part of the gastrointestinal tract, usually the terminal ileum or the large bowel. Most writers mention Bang as having first described this condition in 1876, but the earliest case report has been traced to 1738 (1). More than 200 cases have been described in the world literature. This condition may be asymptomatic and found incidentally during laparotomy or at autopsy. Very few cases have been diagnosed by roentgenography alone, the first such case in the American literature having been reported by Lerner and Gazin in 1946 (2). In 1949, Gazin, Brooke, Lerner, and Price also reported the first American case to be diagnosed preoperatively and confirmed by surgery and histologic study (3). While the presence of pneumoperitoneum which results from the rupture of a cyst is a rather common complication, it is believed that the following case is the first to be diagnosed after establishment of a therapeutic pneumoperitoneum.

CASE REPORT

A 28-year-old Negro male cook was admitted to the Veterans Administration Hospital, Albuquerque, New Mexcio, on June 22, 1953, with a history of productive cough, fever, malaise, and 15 pounds' weight loss of six weeks' duration. The patient appeared acutely ill. The positive physical findings were confined to the lungs and confirmed by a chest roentgenogram (figure 1). There was pneumonic consolidation of the right upper lobe, with scattered soft infiltrates throughout the remainder of the right lung field as well as in the middle third of the left lung field. The sputum was positive for tubercle bacilli on microscopy and culture. As there was no history or physical finding to indicate gastrointestinal pathology, no abdominal roentgenograms were obtained on admission. The administration of 1 gm. of streptomycin intramuscularly, 300 mg. of isoniazid, and 12 gm. of PAS daily was started on the day of admission. Cough and fever subsided in three weeks, and the dosage of streptomycin was then changed to 1 gm. twice weekly.

Due to the persistence of tubercle bacilli in the sputum and the presence of bilateral disease, pneumoperitoneum was induced on February 16, 1954. A chest roentgenogram (figure 2) obtained one month after the initial pneumoperitoneum revealed considerable medial displacement of the liver by air and a loop of cystic bowel lying lateral to this organ. Fluoroscopic examination prior to pneumoperitoneum refill showed the startling picture of multiple conglomerated translucencies along the entire right side of the abdomen. A flat plate of the abdomen (figure 3), barium enema studies (figure 4), and a gastrointestinal series (figure 5) revealed the extent of this condition more clearly.

The patient complained of considerable abdominal discomfort for several days following each pneumoperitoneum refill, which varied between 450 to 600 cc. of air with positive

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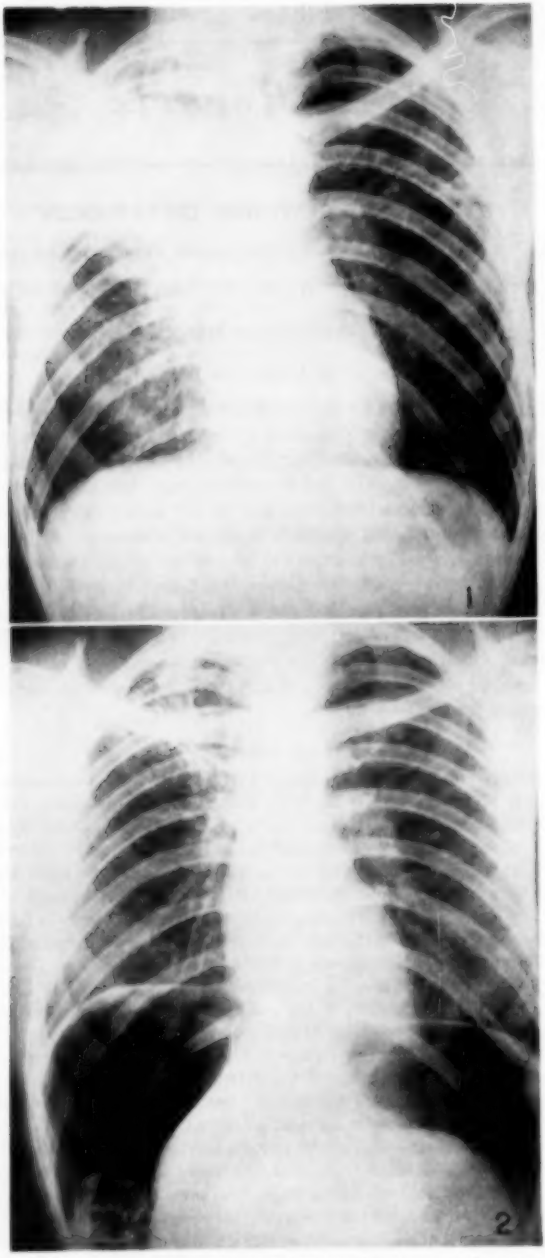

FIG. 1. Initial chest roentgenogram (June 23, 1953), showing massive consolidation in the upper lobe, with soft densities in the lower half of the right lung field and middle third of the left lung field.

FIG. 2. Chest roentgenogram (March 13, 1954), one month after induction of pneumoperitoneum, showing raised loop of cystic hepatic colon.

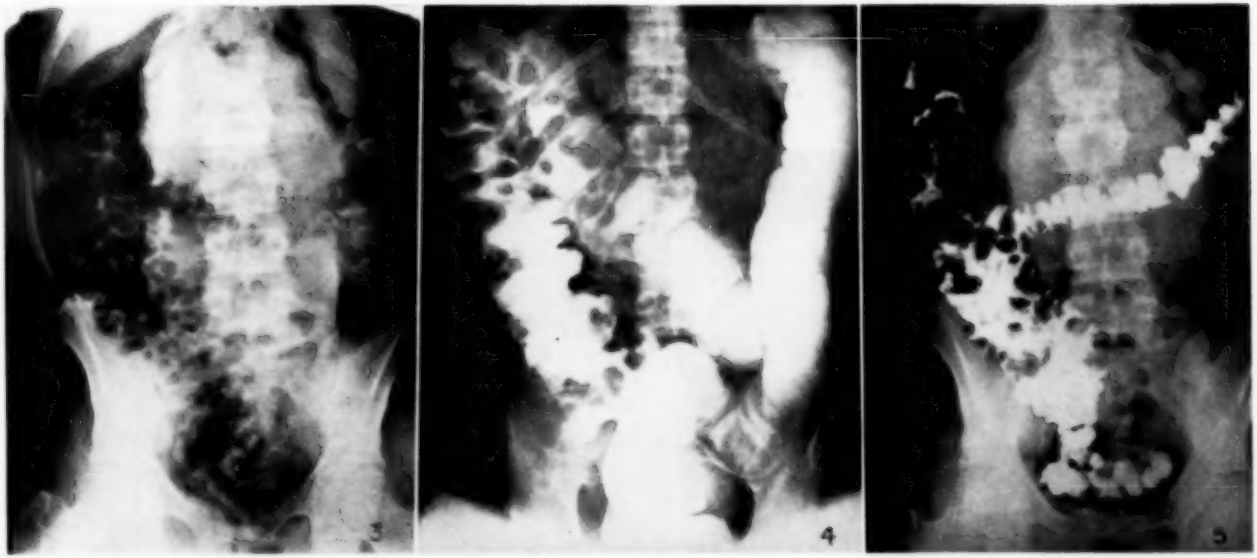

FIG. 3. Flat film of abdomen (July 8, 1954), showing pneumatosis cystoides intestinalis involving the cecum and colon to the mid-descending portion. Multiple thin-walled air-filled cysts of varying size are most numerous in the ascending colon.

FIG. 4. Barium enema studies (August 9, 1954) performed prior to the gastrointestinal series, illustrating both submucosal and subserosal cysts.

FIG. 5. Four-hour film of gastrointestinal series (August 12, 1954) reveals the cysts to be both submucosal and subserosal. Barium is caught between the submucosal cysts in thin lines. Reabsorption of cysts in transverse colon has taken place. No evidence of cysts in terminal ileum.

FIG. 6. Flat film of abdomen two months later (October 12, 1954) reveals absorption of many of the cysts.

FIG. 7. Flat film of abdomen (January 12, 1955) shows complete disappearance of the cysts, only the normal findings of gas within the large bowel being visualized.

pressure of 5 to 10 cm. of water. Pneumoperitoneum refills were discontinued on October 12, 1954, just prior to resection of the right upper lobe.

An abdominal roentgenogram (figure 6) on October 12, 1954, following the final pneumoperitoneum refill, revealed almost complete spontaneous disappearance of the cysts. A recent flat film of the abdomen (figure 7) taken on January 12, 1955, showed no evidence of pneumatosis intestinalis, only the normal findings of gas within the large bowel being visualized.

COMMENT

The cause of pneumatosis cystoides intestinalis is unknown. Several theories have been offered to explain this condition.

Bang and, later, Finney advanced the neoplastic theory, presuming the presence of a gas-producing cell. However, neoplastic cells have not been found on microscopic examination in reported cases (4).

The bacterial theory was also suggested many years ago to account for the gas which was supposedly produced by gas-forming microorganisms. This theory has also been discarded, as the cysts have been sterile in most cases or the flora has been very variable in other cases. Spontaneous pneumoperitoneum has been rather frequently reported, but without accompanying peritonitis, which would tend to disprove the bacterial origin of this disease.

According to some investigators (5), pneumatosis intestinalis may be due to severe dietary deficiency. This theory is based on the development of this disease in the majority of pigs fed on a deficiency diet of polished rice and washed casein. Whole corn, unpolished rice, yeast, and milk tended to prevent the development of this disease. The specific factor in the diet was not determined. Frequent mention is made in reported cases of considerable weight loss, suggesting that some nutritional factor associated with an obstructive lesion may be at least partially responsible for this condition.

The mechanical theory appears to be the most popular and presumes that the gas is forced through the stomach or bowel wall via gross ulcerations or minute abrasions caused by overdistention. Some investigators believe that the gas travels to distant areas in the intestinal tract via the lymphatic system.

Stiennon (6) has advanced a new mechanical theory, believing that the gas, under pressure from the obstructed stomach or other portions of the gastrointestinal tract, dissects through a mucosal defect in the bowel wall into the root of the mesentery. From here it spreads via the mesentery to various layers of the bowel wall, usually some distance from the site of obstruction. Such a mechanism would explain why the duodenum is never involved and also the occasional presence of gas cysts in the mesentery.

The cysts are usually located in the small bowel with the exception of the duodenum, but may be found in the large bowel, and more rarely in the stomach, areolar tissue of mesentery, and in retroperitoneal tissues. The areas most usually involved are between the beginning of the jejunum and the transverse colon. "Skip areas" of normal bowel may be present between involved segments. Gas cysts in the gastrohepatic ligament, gallbladder, falciform ligament, diaphragmatic and parietal peritoneum, and retroperitoneal lymph nodes have also been reported (7). The cysts may occur singly but are usually multiple, forming grapelike clusters in most instances. They are predominantly subserosal in adults, although any or all layers of the bowel wall may be involved. Their size may vary greatly from a millimeter to several centimeters. The cysts may be widely distributed in some cases or localized to a specific portion of the bowel in others. The terminal ileum is a more common site. Typical microscopic findings reveal thin-walled sacs lined with a layer of flat endothelial cells and surrounded by sparse connective tissue with some elastic

fibers. Many cysts show only a partial endothelial lining or none at all. Acute inflammatory cells, small round-cell infiltration, giant cells, and fibrosis in the surrounding wall have also been described, giving the appearance in some cases of a foreign-body and inflammatory type of reaction.

In most cases the cysts contain only gas, but small hemorrhages or serous fluid may be found within the cysts or in the cyst wall. Fibrosis and scarring may occur, with decrease in the size or disappearance of the cysts. Small fibrotic areas may be the only residuals in some instances. Analysis of the gaseous contents of the cysts have yielded varied results. Some investigators have noted that the gas within the cyst was similar in composition to that of atmospheric air, while others have reported a marked dissimilarity in the gases from the two sources. It is generally agreed that the chemical composition of the cyst gas is not comparable with that present in the gastrointestinal tract. Various investigators (8, 9) have determined the cyst content to be nitrogen (70 to 90 per cent), carbon dioxide (0 to 15 per cent), and oxygen (0.5 to 20 per cent). After brief exposure of the cysts to air, gaseous diffusion may occur, altering the original contents.

The majority of cases of pneumatosis cystoides in adults are associated with chronic obstruction of the upper gastrointestinal tract produced by peptic ulcer or carcinoma. When associated with intestinal obstruction, the site of cyst formation may be a considerable distance from the point of actual obstruction. Olson (10) described his operative findings in an eight-year-old girl who had a partial obstruction of the duodenum, apparently caused by a congenital kinking. At operation the cysts were confined to the terminal ileum.

The disease has been invariably fatal in infants, death being due to intestinal obstruction. In contradistinction to the predominantly subserosal location of the cysts in adults, the submucosa in infants is the site of predilection. Sufficient reduction of the bowel lumen by invagination of the mucosa adjacent to the cysts may initiate intussusception, resulting in vomiting, diarrhea, and abdominal distension. In the largest series reported in infants, MacKenzie (8) found a high incidence of abnormalities related to the gastrointestinal tract that were either congenital or acquired during the neonatal period. He also mentions that the only significant physical sign is a crepitant, nontender mass in the abdomen. This sign has been only rarely mentioned in other reported cases. Subserosal cysts do not ordinarily cause symptoms of bowel obstruction and, therefore, the disease in adults generally runs a more benign course. The patients are usually malnourished. Koss (9) found no associated lesion in 15 per cent of reported cases, and he uses the term "primary pneumatosis cystoides intestinalis" for this group.

Roentgenographic findings are very characteristic (11). A plain film of the abdomen shows multiple accumulations of gas, usually outside the intestinal lumen, following the bowel contour. Occasionally submucosal gas may invaginate the mucosa, narrowing the lumen of the bowel. The gas may be localized in the mesenteric or retroperitoneal spaces. If rupture of a cyst has occurred, pneumoperitoneum may be seen in addition to the cystic areas and may lead to the erroneous diagnosis of perforation of a viscus. The presence of gas under the diaphragm with no clinical evidence of a perforated viscus is highly suspicious of pneumatosis cystoides intestinalis.

Gas cysts in the small intestine may cause sufficient buoyancy to allow a loop of cystic bowel to rise into the subdiaphragmatic area on either side, displacing the liver or stomach downward. This appearance may be confused with pneumoperitoneum in the roentgenogram, but the presence of linear bowel markings and the absence of shift of the translucency when the patient is examined in different positions confirms the presence of cystic bowel. Both conditions may be present simultaneously, and then the contrast between

cystic bowel and pneumoperitoneum is more pronounced (figure 2). Rarely, an accumulation of gas occurs between the peritoneal and muscular layers of the diaphragm, also simulating pneumoperitoneum. It should be noted that, in all instances of pneumatosis cystoides intestinalis, the gas is extraperitoneal in location unless rupture of a cyst occurs.

Intestinal obstruction is the most serious complication of pneumatosis intestinalis. It may occur from encroachment into the bowel lumen from the cysts themselves or may be found some distance proximal to the cystic area due to associated conditions, usually in the vicinity of the pylorus. The obstruction usually requires surgical relief with possible resection of the cystic area. Cases in which gas cysts are widespread are not suitable for excision. Puncture of the cysts is usually not possible, as many are tiny and there may be thousands. When the cysts are localized to a small segment of bowel, resection may be done to prevent possible later obstruction (12). In some cases following surgical relief of the obstruction, subsequent re-exploration of the abdomen has shown disappearance of the cysts. If pneumatosis intestinalis is an incidental finding and the patient is asymptomatic, no specific treatment is necessary. The blebs may diminish in size spontaneously or disappear altogether in a few weeks or months.

SUMMARY

A case of pneumatosis cystoides intestinalis is presented. While pneumoperitoneum has been reported as a complication secondary to rupture of a cyst, it is believed that this is the first case diagnosed after induction of a therapeutic pneumoperitoneum.

It is suggested that particular attention be paid to abdominal organs during fluoroscopic examination prior to the first few therapeutic pneumoperitoneum refills, as the air introduced may serve as a diagnostic pneumoperitoneum as well.

SUMARIO

Neumatosis Cistoidea Intestinal: Hallazgo Fortuito Durante el Neumoperitoneo Terapéutico

Preséntase un caso de neumatosis cistoidea intestinal. Aunque se ha descrito neumoperitoneo como complicación secundaria al desgarro de un quiste, parece que éste es el primer caso diagnosticado después de la inducción de un neumoperitoneo terapéutico.

Sugérese que se preste atención en particular a los órganos abdominales durante el examen fluoroscópico antes de las primeras reinsuflaciones del neumoperitoneo terapéutico, pues el aire introducido puede servir igualmente como neumoperitoneo diagnóstico.

RESUME

Pneumatose kystique de l'intestin: sa découverte accidentelle au cours de la création d'un pneumopéritoine thérapeutique

Un cas de pneumatose kystique de l'intestin est présenté. Bien que l'apparition du pneumopéritoine, comme complication secondaire de la rupture d'un kyste ait été rapportée, ce cas para't être le premier cas diagnostiqué après l'instauration d'un pneumopéritoine thérapeutique.

Les auteurs suggèrent d'accorder une attention particulière à l'examen des organes abdominaux au cours de la radioscopie précédant les premières réinsufflations du pneumopéritoine thérapeutique, car l'air introduit peut également servir de pneumopéritoine diagnostique.

Acknowledgment

The writers wish to thank Mr. Howard Toenniges, Chief, Medical Illustration Laboratory, for the photographic illustrations.

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MALIGNANT THYMOMA ASSOCIATED WITH MYASTHENIA GRAVIS[1]

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(Received for publication March 28, 1955)

In 1901, Weigert (1) first reported a case of myasthenia gravis associated with a thymoma. It was not until after 1935, however, that more frequent reports began to appear in the literature. Nevertheless, Hurxthal and Musulin (2) state that the disease remains rare and report an incidence of one case in 60,000 general clinical admissions. It has been reported that approximately 50 to 75.6 per cent of patients with myasthenia gravis have an enlargement of the thymus and 15 per cent of patients with thymomas have myasthenia gravis (3, 4). Malignant thymoma associated with myasthenia gravis is very rare. Crosby (5), in 1932, reviewed all of the cases of malignant thymic tumors published up to that time and found a total of 166 cases. Of these 166, only 2 had associated myasthenia gravis. Since this time, approximately 100 cases of malignant thymoma have been published and only 3 of these patients had associated myasthenia gravis (6, 7). The present case is an interesting case of myasthenia gravis with an associated malignancy of the thymus gland in a patient whose symptoms improved remarkably after surgery.

CASE REPORT

The patient was a 34-year-old white female, first seen by the writers on August 26, 1954, who complained of an inability to shut her mouth and difficulty in masticating and swallowing of six weeks' duration. There was no history to suggest compression of mediastinal structure. Two days previously she had noticed a drooping of the left eyelid which was more noticeable in the evening than in the morning. She denied any pain, numbness, or weakness in her extremities. She denied double vision. In the past the patient had been healthy and strong without any significant illness. She had gone through two pregnancies uneventfully within the past three years.

On initial examination there was an evident jaw drop, which required closing by hand, and a moderate inability to smile. A ptosis of the left eyelid was present. On gaze to the left there was an inconstant diplopia. Examination of the eyes revealed normal fundi and no visual field defects. The tongue was normal. No weakness or fatigability of extremity muscles could be demonstrated. There were no reflex or sensory changes. Examination of the neck was normal. There was no palpable thyroid, and hyperthyroidism was not evident clinically. Examination of the chest revealed no abnormalities on auscultation and percussion. The heart was normal to physical examination, with a normal sinus rhythm of 84 and a blood pressure of 110 systolic, 60 diastolic in mm. of mercury. The heart did not appear to be enlarged and no thrills or murmurs were noted. Abdominal examination revealed no abnormalities. Neurologic examination likewise revealed no abnormalities aside from the above-mentioned weakness and diplopia.

A diagnosis of bulbar myasthenia gravis was suspected, and the patient was given 4.5 mg. of neostigmine and 0.6 mg. of atropine intramuscularly. The medication was followed by a remarkable improvement and disappearance of the signs of weakness of the jaw musculature and total clearing of the ptosis. The patient was placed on neostigmine hydrobromine, 15 mg. tablets, four times a day until the diagnostic studies could be completed. Improvement was marked during this time. The initial impression of bulbar myasthenia gravis was confirmed.

Complete blood counts and urinalyses yielded normal values. A determination of the uptake of radioactive iodine was within normal limits. A chest roentgenogram and fluor-

[1] From the Department of Internal Medicine, Goddard Clinic, Brockton, Massachusetts.

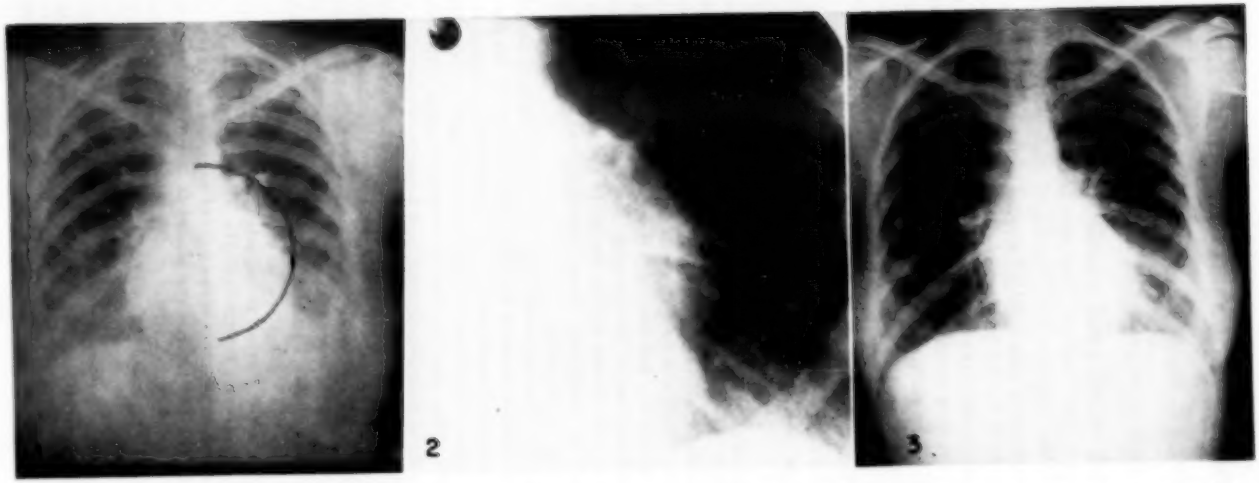

FIG. 1. Preoperative postero-anterior roentgenogram of the chest showing irregular, ovoid density overlying the left border of the heart. Semicircle represents area of cone down, as shown in figure 2.

FIG. 2. Cone down of area shown in semicircle of figure 1. The ovoid mass is clearly seen along the left border of the heart.

FIG. 3. Postoperative postero-anterior roentgenogram of the chest. The left border of the heart now appears normal.

oscopy revealed normal lung fields with a normal-sized heart and aorta. There was an irregular, somewhat ovoid, density overlying the left border of the heart which did not pulsate (figures 1, 2, and 3). Further studies revealed that this was not connected to the esophagus or bony thorax. In view of the onset of myasthenia gravis and the knowledge that thymic tumors are seen in 50 to 75.6 per cent of the myasthenia gravis cases, it was postulated that this was a thymic tumor in an uncommon location.

Bronchoscopy was done and the larynx, trachea, and bronchial carina were found to be normal. Both bronchial trees were normal throughout. There was no evidence of compression or abnormalities. No significant secretions were seen.

A thoracotomy was performed and good exposure of the anterior mediastinum was obtained by using a submammary incision sectioning the third and fourth costal cartilages and placing the Finochietto rib spreader in place. The left pleural space was free of adhesions and fluid. Presenting immediately into the wound in the anterior mediastinum and adherent to the anterolateral surface of the pericardium over the area of the left atrium was a well-encapsulated, irregularly nodular mass of moderate firmness which was slightly mobile over its deep attachments. This mass was very easily dissected off the pericardium and from adjacent fatty tissue and was adherent by its superior pole attachment to the thoracic duct, in which there were some remnants of apparently normal thymus tissue. The tumor mass was close, but not attached, to the phrenic nerve on the left. In removing some portions of the normal-appearing thymus tissue, which extended toward the right pleural space, a small opening in the right pleural sac was made. This was controlled by placing a small catheter in the right pleural space. Superior mediastinal lymph nodes along the course of the phrenic nerve were present in the surrounding fatty tissue and these were removed. The diaphragm was active and mobile. Both lobes of the lung inflated and deflated normally and were normal to palpation. The patient tolerated the operation well. There was no gross evidence of invasion of this tumor into any of the adjacent tissues. The patient's recovery was excellent, and no further neostigmine has been necessary. At the present time,[1] nine months after the operation, the patient is caring for her husband and children and is receiving no medication. She experiences mild jaw drop only after an unusually hard day with her children. She does all of her own housework without difficulty, and the above-mentioned jaw weakness has occurred only occasionally and has not been severe enough to warrant medication.

The specimens removed were sent to the pathology laboratory. The first was a small oval piece of yellow tan tissue 1 cm. in length and soft in consistency. The second was a 4 by 2 by 1 cm. ragged soft tan-pink tissue mass which was partially covered by smooth, transparent serosal lining. On sectioning, it showed that it was composed of firm, yellow-white tissue. Microscopic examination of the sections revealed hypertrophied thymus. The third specimen was an ovoid, flattened mass enclosed in a thin transparent capsule, weighed 120 gm., and measured 11 by 7 by 3 cm. The outer surface was irregularly nodular, being studded with smooth, soft nodules, varying in diameter from approximately 0.5 to 3 cm.

Cut sections showed the mass to be composed of soft white encephaloid tissue with scattered patches of hemorrhage. Included with this specimen were several soft yellow lymph nodes. Microscopic examination of this mass revealed it to be a malignant thymoma of the lympho-epithelioma type. The accompanying lymph nodes were considered normal.

COMMENT

When the diagnosis of myasthenia gravis is established, it is important to obtain radioactive iodine uptake studies, chest fluoroscopy, and chest roentgenograms taken with the patient in various positions. Thymic neoplasms, when present, are usually found to be retrosternal, but thymomas have also been found at the level of the ninth or tenth thoracic vertebra, in front of the pericardium, above the diaphragm, or on the anterior surface of the left main stem bronchus (8).

[1] March 25, 1955.

There is no way to determine by roentgenography whether a thymic mass is malignant or benign and, indeed, review of the literature is most confusing as to the exact pathologic criteria for malignancy in certain cases. Fisher and associates (6) and Seybold and coworkers (3) pointed out quite definitely that one cannot make the diagnosis of malignant or benign thymoma in all cases on a histologic basis alone. Jones (9) lists the following points indicating the thymic origin of mediastinal sarcoma:

1. The situation of a large, slightly lobulated tumor at the site of the thymus
2. The extension of this tumor downward behind the sternum without infiltration of the bone
3. The involvement of the pericardium and of the pleura by direct lymphatic extension
4. The resemblance to thymic tissue on histologic examination

The present case satisfies the identifying features for the diagnosis of malignant thymoma. When these identifying features cannot be met, suspected benign thymic tumors must be regarded with suspicion.

There are no histologic criteria which enable one to differentiate thymic neoplasms associated with myasthenia gravis from those unassociated with the disease (6). Patients with myasthenia gravis and demonstrable mediastinal masses should be subjected to thoracotomy and excision of the mass.

Treatment of thymic tumors by radiation deserves mention. Thymus carcinoma is radio-resistant; whereas, lymphoid tumors of the thymus are often strikingly radiosensitive, although occasionally radio-resistant (10, 11). Radiation therapy may have value as a postoperative procedure but should not take precedence over thoracotomy and excision in operable cases. Fortunately, the patient of the present case has required no further neostigmine since her surgery. There has been no clinical or roentgenographic evidence of metastasis.

SUMMARY

A case of myasthenia gravis associated with malignant thymoma is presented. The case satisfies the identifying features for the diagnosis of malignant thymoma. When these identifying features cannot be met, suspected benign thymic tumors must be regarded with suspicion.

SUMARIO

Timoma Maligno Asociado con Miastenia Grave

Preséntase un caso de miastenia grave asociada con timoma maligno. El caso cumple las características de identificación para el diagnóstico de timoma maligno. Cuando no pueden satisfacerse esas características de identificación, hay que considerar con sospecha los tumores tímicos benignos dudosos.

RESUME

Thymome malin associé a la myasthénie grave

Présentation d'un cas de myasthénie grave associée à un thymome malin. Ce cas possède les caractéristiques permettant le diagnostic de thymome malin. Quand ces signes caractéristiques font défaut, la malignité des tumeurs bénignes du thymus paraissant suspectes peut être mise en doute.

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Notes

EFFECT OF DEGREE OF HEALING UPON PERSISTENCE OF TUBERCLE BACILLI WITHIN PULMONARY LESIONS

Previous reports from this laboratory have indicated that, in the vast majority of instances, tubercle bacilli remain viable within necrotic foci in patients who have received prolonged courses of chemotherapy.[1-3] No correlation was noted, however, between the frequency with which viable cells were detected and the following factors:

1. the duration of preoperative chemotherapy
2. the total duration of chemotherapy
3. the preoperative chemotherapeutic regimens employed
4. the duration of the preoperative period of noninfectiousness
5. the morphologic nature of the lesion, i.e., the degree of closure of the cavity

An important question thus arose. When any single microbiologic procedure capable of detecting persisting tubercle bacilli with a high degree of frequency is employed, from what types of patients and/or lesions can they be recovered? In other words, under *what conditions* do tubercle bacilli survive? The present report describes certain observations made in an attempt to answer these questions.

Material used: Sixty-five morphologically "closed" tuberculous lesions have been included for study. The source and nature of these lesions have been described in detail in a previous communication.[2, 3]

* * *

Anatomic course of the disease: For the purpose of the present investigation, the inspissated cavity has been grouped together with the necrotic focus which has never liquefied. This is justified by the fact that a tuberculous cavity, when it closes with inspissation of its necrotic contents, is converted into a necrotic focus. The inspissated cavity shows a fragmentation of the necrotic tissue with a loss of elastic fibers, while the necrotic focus which has never liquefied shows an intact elastic framework.

The development and healing of a tuberculous process in the lung generally occur in a definite and orderly manner. The lesion which occurs in the lung in an adult in most instances appears in a definite location, namely, the upper lobe in the posterolateral aspect approximately 2 cm. above the interlobar fissure.

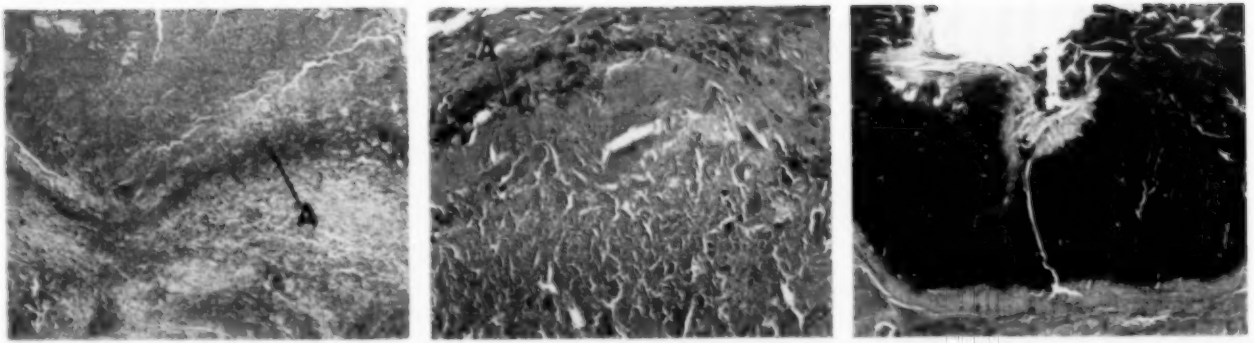
The first change in the lung is the development of a bronchopneumonia which rapidly undergoes necrosis. As necrosis occurs, an area of granulation tissue forms around the tuberculous pneumonia. At first this zone is rich in cells (fibroblasts, epithelioid, and Langhans' giant cells), the capillaries are dilated, but few collagen fibrils are present. The tuberculous pneumonia may undergo liquefaction, and, with evacuation of its contents, form a tuberculous cavity with necrotic fragments lining the inner wall. The zone of granulation tissue lies beyond the necrotic layers, and, when cavity closure occurs, the necrotic fragments which fill the space are encircled by this zone of granulation tissue.

As the process of healing continues, the collagen fibrils which are laid down by the fibroblasts and epithelioid cells increase in number, with a corresponding decrease in the number of capillaries and cells. This continues until the capsule is composed of dense

[1] Hobby, G. L., *et al.*: Am. Rev. Tuberc., 1954, *70*, 191.

[2] Hobby, G. L., *et al.*: Tr. Fourteenth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1955 (in press).

[3] Hobby, G. L.: Am. J. Med., 1955, *18*, 753.



1 2 3

FIG. 1, Stage 1. Early healing. The necrotic center is surrounded by a layer of granulation tissue (*A*).

FIG. 2, Stage 2. Intermediate degree of healing. The necrotic zone is surrounded by a wide area of hyalinized connective tissue containing coal pigment.

FIG. 3, Stage 3. Advanced healing. The necrotic center is almost completely occupied by calcium deposits and is surrounded by a hyalinized connective tissue capsule.

hyaline connective tissue. As the area of granulation tissue is converted into hyalinized connective tissue, there is a contraction of the capsule with a compression of the necrotic center. Fluid is absorbed from the area of necrosis which thus becomes drier and firmer. Cholesterol deposits in varying amounts are first observed in the necrotic zone with the development of hyalinization in the capsule. Calcium salts appear in the area of necrosis as the process of hyalinization is complete or nearly complete. Calcium is first deposited in the necrosis at its periphery as a fine stippling and, in the early stages, may be mistaken for nuclear fragments.

Deposition of calcium salts may continue until it occupies the entire necrotic zone. Occasionally calcium deposition occurs in the form of concentric layers (Liesegang rings) parallel to the capsule.

Anatomic age of lesions: Inspissated tuberculous cavities and necrotic foci which have never liquefied have been arbitrarily divided into three groups, based on the degree of healing observed:

Stage 1—Early healing: The lesion is surrounded by a layer of granulation tissue. Although parts of the wall may show hyalinization, the cellular and vascular elements of the granulation tissue predominate. The necrotic zone contains little or no calcium.

Stage 2—Intermediate degree of healing: The lesion shows advanced hyalinization of the capsule without calcium deposits within the necrotic zone. Cholesterol may be present at times.

Stage 3—Advanced healing: The lesion is surrounded by a layer of hyalinized connective tissue in which cellular elements are rarely seen. The necrotic center contains large amounts of calcium salts.

* * *

Correlation of anatomic age of lesions with microbiologic observations: As mentioned previously, 65 morphologically "closed" necrotic lesions have been studied to date.

Tubercle bacilli have been recovered from *all* of 29 lesions showing early healing (stage 1) and from 6 of 14 lesions showing an intermediate degree of healing (stage 2). In contrast, *M. tuberculosis* has been recovered from only 3 of 22 lesions showing advanced healing (stage 3).

Two of the last-mentioned 3 lesions from which tubercle bacilli were recovered had been removed from a patient who had received only seven days of chemotherapy preoperatively; the third had been removed from a patient who had received isoniazid and para-aminosalicylic acid for five months preoperatively and yielded organisms highly resistant to the action of isoniazid.

* * *

The data presented herein are in agreement with the views of Canetti[4, 5] who, in a recent review of the literature, called attention to the fact that the frequency with which tubercle bacilli may be recovered from "closed" caseous lesions decreases as the signs of activity diminish and the caseous material becomes more solid. Hall and Gleason,[6] moreover, have likewise observed a decrease in the frequency with which tubercle bacilli may be recovered from nodules in proportion to the histologic signs of activity. The lessening of

[4] Canetti, G.: Bull. Internat. Union Against Tuberc., 1954, *24*, 144.

[5] Canetti, G.: The Tubercle Bacillus in the Pulmonary Lesion of Man, Springer Publishing Co., Inc., New York, 1955.

[6] Hall, W. H., and Gleason, D. F.: Tr. Twelfth Conference on the Chemotherapy of Tuberculosis, Veterans Administration, Army, and Navy, 1933, p. 219.

activity is manifested morphologically by hyalinization of the capsule and deposition of calcium in the necrotic center.

According to Canetti, the frequency with which tubercle bacilli may be recovered from "closed" tuberculous lesions varies from 72 per cent in recent or softened nodules to 0 per cent in chalky or calcified nodules. In the experience of the present investigators, it has varied from 100 per cent to 12 per cent in these two groups, respectively.

Mention has been made previously of the fact that no correlation has been noted between the frequency with which viable cells have been detected in "closed" pulmonary lesions and the duration of preoperative chemotherapy.[1] Canetti in like manner has observed no such correlation, but has called attention to the fact that this does not exclude the possibility that chemotherapy may play a part in changing the state of a lesion from that of caseation, by collagenous replacement, into that of fibrous tissue. Nor is the possibility excluded that chemotherapy may play a part in changing an open caseous lesion, i.e., a cavity, into a "closed" caseous lesion. Although chemotherapy per se may not be capable of eradicating each and every infecting microorganism from a tuberculous lesion, it may act indirectly to bring about this effect.

It is of interest to note in this regard that, in the opinion of one of the present authors (O. A.), the prolonged use of currently available chemotherapeutic agents (streptomycin, isoniazid, para-aminosalicylic acid) shortens the period of time required for healing of tuberculous foci, and calcification occurs in a shorter period of time with than without chemotherapy. In his experience, calcified tuberculous foci are observed more frequently now than in the era prior to chemotherapy. The evidence to support the opinion that chemotherapy speeds the healing process will be the subject of a forthcoming communication by this investigator (O. A.).

The possibility that chemotherapeutic agents and regimens other than those commonly used today may further hasten the healing process, or may effect total eradication of all infecting microorganisms prior to completion of healing, i.e., prior to calcification, warrants consideration.

In summary: The anatomic changes which occur during healing of a tuberculous lesion have been described. Data have been presented to indicate that the frequency with which tubercle bacilli may be recovered from "closed" necrotic lesions is directly related to the anatomic age of the lesion.

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THE INFLUENCE OF THE SIZE OF INOCULUM ON SUSCEPTIBILITY TESTING OF MYCOBACTERIUM TUBERCULOSIS

The question of the importance of the size of inoculum in drug-susceptibility tests with *M. tuberculosis* has been noted previously. For example, it has been observed that the concentration of para-aminosalicylic acid (PAS) in a liquid medium necessary to inhibit growth varied considerably with the size of inoculum. Thus, Vennesland and associates[1] found that more than one hundred times the amount of PAS was necessary to cause inhibition if an inoculum of 1.0 mg. of tubercle bacilli per 10 ml. of media was used instead of an inoculum of 0.1 mg. of bacilli. Youmans and associates[2] observed that the bacteriostatic action of PAS was inversely proportional to the number of tubercle bacilli present in the medium. On the other hand, numerous observers reported that the size of inoculum does not influence the susceptibility of *M. tuberculosis* to streptomycin. Youmans[3] observed that the bacteriostatic action of streptomycin for *M. tuberculosis* was not significantly affected by an inoculum within the range of 0.1 mg. to 1.0 mg. Williston and Youmans[4] found that an inoculum of 0.01 mg. to 0.25 mg., wet weight, did not influence the results of their streptomycin-susceptibility tests, provided that a minimum incubation period of twenty-eight days was employed, since faster growth was observed with larger inocula. Robinson and associates[5] found that an inoculum with an even wider range of 0.1 mg. to 3.0 mg. had almost no effect on the results of their drug-susceptibility tests.

A comparative study of the standard method of drug-susceptibility testing of *M. tuberculosis* on solid media was undertaken with a new modification using different concentrations of inoculum.

The routine method used was originally described by Steenken[6] and recommended by the American Trudeau Society (ATS) Subcommittee on Laboratory Methods[7] (also approved by the Twelfth Veterans Administration-Army-Navy Conference on the Chemotherapy of Tuberculosis[8]). The modified method was essentially the same as the standard one except that, in addition to the original set of drug-containing tubes cultured with an undiluted inoculum of *M. tuberculosis*, a series of tubes using serial dilutions of 10^{-1} to 10^{-7} of inoculum were introduced. The undiluted inoculum used in this laboratory contained 2.5 mg. to 3.7 mg. of tubercle bacilli, wet weight, by the Hopkins vaccine centrifuge tube, per culture tube. The inoculum was prepared by emulsifying the tubercle bacilli in an emulsifier used routinely in this laboratory.[9]

American Trudeau Society medium was used for both controls and drug-containing

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[9] Kenney, M.: Veterans Administration Management Improvement Bulletin, 1953, MIB4, 10.

tubes. The drugs used were streptomycin, PAS, and isoniazid. Both streptomycin and PAS were incorporated in the medium in concentrations of 10 and 100γ per ml. while isoniazid was used at concentrations of 0.2, 1.0, and 5.0γ per ml.

The organisms studied were typical strains of *M. tuberculosis* subcultured on ATS medium from the original cultures isolated from the patients. The cultures were examined once a week for a total of eight weeks.

One hundred and eighty-nine drug-susceptibility tests, with a total of 1,512 cultures, were performed. Among the 189 tests, 50 showed complete susceptibility of the tested strains of tubercle bacilli to all three drugs, i.e., only the control tubes contained a 3- to 4-plus growth of bacilli. Table 1 demonstrates the number of tests performed and the results obtained with the different concentrations of drugs used. Table 2 and figure 1 represent the number of positive tests obtained with diluted and undiluted inocula. The end point in reading results was the presence of growth without taking into consideration the relative density of the growth as compared with the control drug-free cultures. In the majority of the tests, however, in which growth in the drug-containing tubes was obtained with higher dilutions of inoculum, the density of growth was comparable with that of the controls.

TABLE 1

NUMBER OF TESTS PERFORMED AND THE RESULTS OBTAINED WITH DIFFERENT CONCENTRATIONS OF DRUGS USED

Drugs	Concentrations	Total	Susceptible	Resistant
	γ per ml.			
Streptomycin	10	28	5	23
Streptomycin	100	21	5	16
PAS	10	18	4	14
PAS	100	15	5	10
Isoniazid	0.2	26	5	21
Isoniazid	1.0	43	12	31
Isoniazid	5.0	38	14	24
Totals		189	50	139

TABLE 2

TESTS SHOWING DRUG RESISTANCE OBTAINED WITH DIFFERENT DILUTIONS OF THE INOCULA

	Tests Showing Drug Resistance				
Drug Concentration	Undiluted Inocula	1:10–1:100 Inoculum	1:1,000–1:10,000 Inoculum	Higher Dilutions	Total
γ per ml.					
Streptomycin 10	6 (26.1%)	5 (21.7%)	2 (8.7%)	10 (43.5%)	23
Streptomycin 100	5 (31.2%)	5 (31.2%)	1 (6.4%)	5 (31.2%)	16
PAS 10	4 (28.6%)	4 (28.6%)	0 (0.0%)	6 (42.8%)	14
PAS 100	5 (50.0%)	2 (20.0%)	1 (10.0%)	2 (20.0%)	10
Isoniazid 0.2	6 (28.6%)	3 (14.3%)	1 (4.7%)	11 (52.4%)	21
Isoniazid 1.0	14 (45.2%)	6 (19.3%)	1 (3.2%)	10 (32.3%)	31
Isoniazid 5.0	11 (45.8%)	5 (20.8%)	0 (0.0%)	8 (33.4%)	24
Totals	51 (36.7%)	30 (21.6%)	6 (4.3%)	52 (37.4%)	139

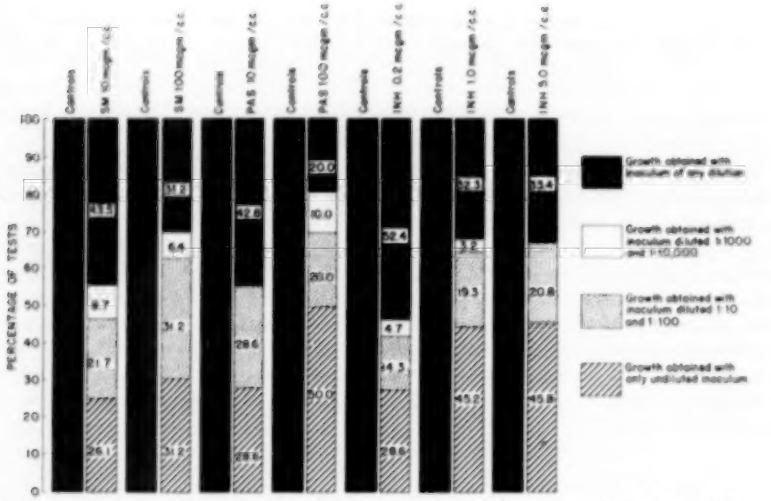
Controls
SM 10 mcgm / c.c.
SM 100 mcgm / c.c.
PAS 10 mcgm / c.c.
PAS 100 mcgm / c.c.
INH 0.2 mcgm / c.c.
INH 1.0 mcgm / c.c.
INH 5.0 mcgm / c.c.
PERCENTAGE OF TESTS
Growth obtained with inoculum of any dilution
Growth obtained with inoculum diluted 1:1000 and 1:10,000
Growth obtained with inoculum diluted 1:10 and 1:100
Growth obtained with only undiluted inoculum

FIG. 1. Percentage of *M. tuberculosis* resistant to antituberculous drugs when different dilutions of inocula were employed.

Among the 139 tests which showed growth in the presence of the drugs used, 51 (36.7 per cent) showed growth only with the undiluted inoculum, while 30 (21.6 per cent) showed growth with inocula diluted 1:10 to 1:100. In 6 (4.3 per cent) of the tests with dilutions of 1:1,000 to 1:10,000 and 52 (37.4 per cent) of the strains, drug-resistance showed with higher dilutions of the inoculum of tubercle bacilli.

Thus, when all other factors such as media processing and the duration of incubation, et cetera, were strictly controlled, the size of the inoculum of tubercle bacilli did in fact influence the final results for all three drugs.

In table 2 and figure 1, it is demonstrated that, in 20.0 per cent to 52.4 per cent of the drug-susceptibility tests, drug-resistant tubercle bacilli were found by using any dilution of inoculum. It is obvious that in this group a test using a single inoculum, diluted or undiluted, would reveal the presence of drug-resistant tubercle bacilli.

On the other hand, in 26.1 to 50.0 per cent of the tests, growth was obtained with only the undiluted inoculum of bacilli. In this group, the standard test, using an undiluted inoculum, revealed these organisms to be drug-resistant, whereas a test using a dilution of the inoculum showed them to be drug-susceptible.

Because the inoculum dilution method is far more cumbersome and time-consuming than the standard test, it would be advantageous to use a single dilution of inoculum in routine drug-susceptibility testing. Only correlation with clinical data will establish which size of inoculum of tubercle bacilli most closely approximates the drug-susceptibility of the tubercle bacilli *in vivo*.

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AN IN VIVO METHOD OF CULTURING TUBERCLE BACILLI: THE CHAMBER METHOD

In order to dermine the significance of the "humoral factor" in the natural and acquired resistance to tuberculosis, a special experimental method is needed. *In vitro* experiments are not adequate for this purpose because body fluids, such as serum or lymph, may change their characteristics on removal from the body and participate in reactions different from those which take place within the body. Accordingly, a new experimental method was planned in order to observe the *in vivo* tuberculostatic powers of body fluids completely separated from tissue cells. By this method it is possible to study the role of the "humoral factor" completely separated from that of cell functions. In the present paper the technique is described in detail.

Ordinary chamber method: A small square plastic plate is prepared (as shown in figure 1) with a larger hole drilled in the center and a smaller hole in each corner. The size of the plate differs according to the animals tested, but in rabbits a 2 by 2 cm. plate is usually used. It is convenient to have a number engraved on each plastic plate.

Crude parchment paper and ordinary filter paper are placed on this plastic plate and bound tightly with threads which pass through the smaller holes. This is used as a filter membrane. This filter membrane is placed in a Petri dish and sterilized at 80°C. overnight. At temperatures higher than 80°C., the plastic plate may melt.

A small watch glass (a little smaller than the plastic plate in diameter) is cleaned with water and sterilized by dry heating in a Petri dish. On the inner surface of this sterilized watch glass, a droplet of suspension of tubercle bacilli treated by the benzene method, described below, is smeared. After evaporation of benzene the watch glass is filled with physiologic saline solution. The sterilized filter membrane is placed on this watch glass (air bubble must not remain in the chamber) and bound tightly with threads as shown in figure 1. Thus, a chamber (chamber *O*) is completed. All manipulations are made aseptically by using sterilized rubber gloves.

The chamber is implanted into the peritoneal cavity of an animal by surgical procedure. Ordinarily six to eight chambers are implanted in each rabbit. Twenty or thirty days after implanting, the chamber is taken out and separated by cutting the threads. The protein concentration of the fluid in the watch glass is determined by a refractometer and some of the fluid is smeared on glass slides. The watch glass is placed upright at room temperature. After drying it is fixed with 95 per cent ethanol for approximately five minutes. The watch glass and the smeared slides are stained by the Ziehl-Neelsen method.

Benzene method: A clump of tubercle bacilli (three weeks' growth in Sauton medium) is placed in a test tube, 2 to 3 ml. of benzene are added, and the tube is shaken. In a few seconds the clump will disintegrate and the supernatant fluid will be turbid. After slight centrifugation (1,000 r.p.m. for five minutes) there is almost no bacillary aggregation, but separate distinct bacilli are noted in the supernatant benzene. The density of these bacilli may be regulated by adding more benzene. A loopful of this supernatant fluid is dropped upon a watch glass. The benzene spreads over the glass surface and evaporates in a few seconds, and the bacilli are distributed as single bacilli and are fixed tightly on the surface.

This method is very simple and, as the distribution of bacilli is equal, the decision whether the bacilli have multiplied or not is very easy.

In the peritoneal cavity, cross-diffusion of body fluid and saline solution through the filter membrane occurs.

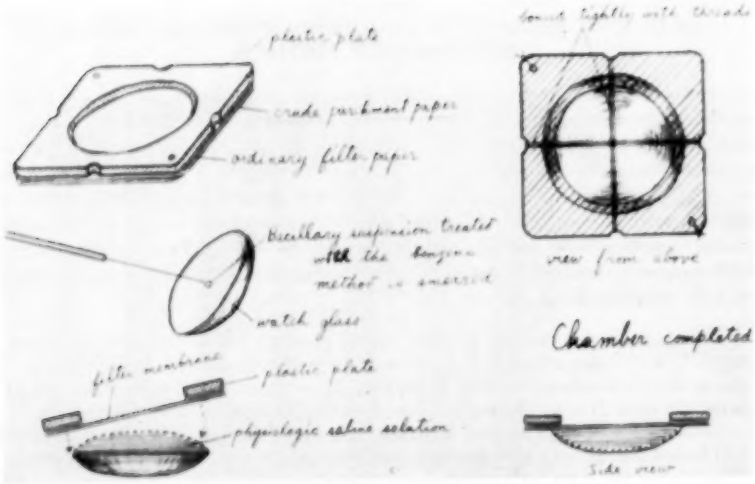

FIG. 1. Diagram of construction of chamber

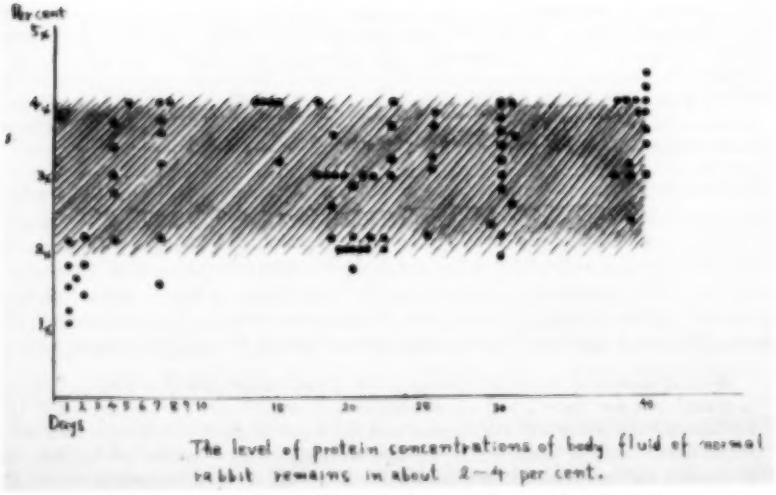

FIG. 2. Protein concentrations of the contents in the chambers

As a preliminary experiment, the following examinations were performed: One, three, five, twenty, and thirty days after the implanting, the chambers were taken out and the protein concentrations of the contents of the chambers were examined by the refractometer. These data are shown in figure 2.

The protein concentrations increased day by day and reached the level of the protein concentration of normal rabbits' peritoneal fluid on the fifth day and after that time

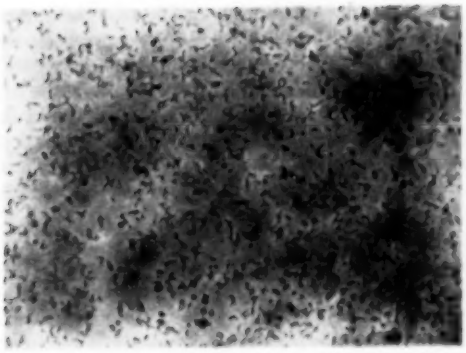

FIG. 3. Proliferation of bovine *RM* tubercle bacilli in rabbit. (× 80)

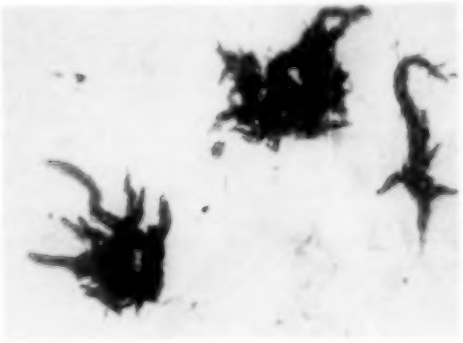

FIG. 4. Proliferation of H37Rv tubercle bacilli in rabbit. (× 1500)

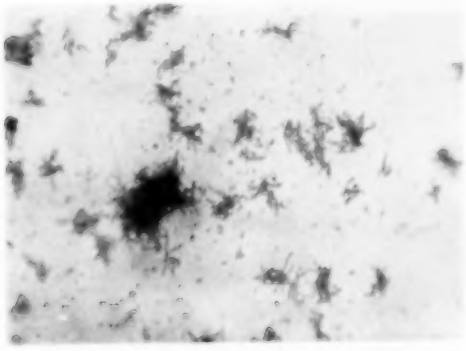

FIG. 5. Proliferation of BCG in guinea pig. (× 1500)

maintained almost the same values. Thus, it is certain that the contents of the chambers become the same as body fluid several days after the implanting. After that time the cross-diffusion of the contents and body fluid takes place smoothly for approximately one month. In cases of very active proliferation of bacilli, however, the protein concentration of the contents increases at a late period beyond the level of that of normal body fluid.

After twenty or thirty days or more, no host cells were found in the chamber. Thus, soluble components as large as protein molecules were allowed to pass through the filter membrane, but none of the particulate matter in the body fluids. When the implantation is not correctly done (when the packings of the chambers are loose), a few or many phagocytic cells may invade the chambers. These chambers were excepted from the experimental results but, in these cases, it is possible to observe the relationship between the host cells and the tubercle bacilli. After sufficient experience had been obtained with the technique, most preparations were satisfactory and no phagocytic cells were seen.

By this method it is possible to cultivate tubercle bacilli as a type of slide-culture method within the living animal body, especially in a medium which contains no tissue cells.

In figures 3, 4, and 5, the proliferations of tubercle bacilli in the chambers implanted

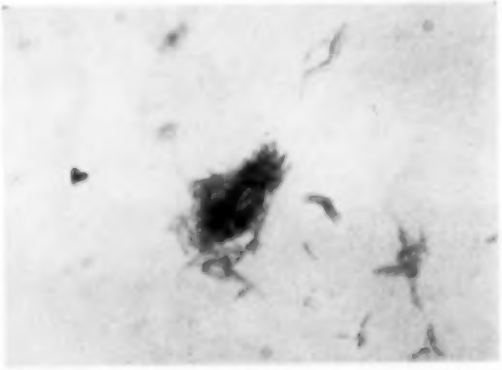

FIG. 6. Proliferation of avian tubercle bacilli (Chamber *S*) in rabbit, after fifteen days. (× 1500)

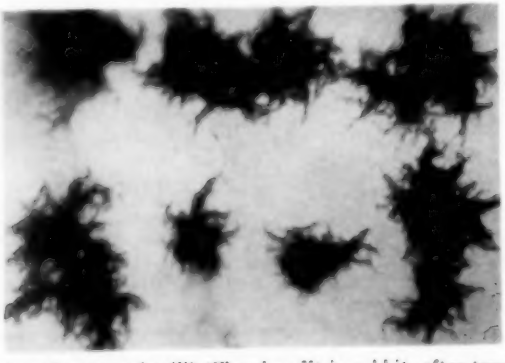

FIG. 7. Proliferation of smegma bacilli (Chamber *K*) in rabbit, after twenty days. (× 80)

into the peritoneal cavities of normal animals are shown. Bovine *RM* bacilli (very highly virulent for rabbits) proliferate very well. Aoyama *B* (human) bacilli and H37Rv proliferate well, but a little less than the *RM* strain. BCG strain can grow in guinea pigs.

Cellophane chamber method: It was possible to culture tubercle bacilli within the living body, especially in a medium which contained no tissue cells, by the chamber method *O*. Moreover, it was concluded that the virulence of tubercle bacilli parallels the ability of bacilli to proliferate in body fluid. To analyze further this influence of body fluid on the growth of tubercle bacilli, the following experiment was performed.

> A chamber was made using cellophane membrane (cellophane No. 300) in place of the crude parchment paper of chamber *O*. This chamber is called the cellophane chamber. The contents of this chamber is the same as that of chamber *O*—physiologic saline solution. This cellophane chamber containing saline solution is called cellophane chamber *S*. In the peritoneal cavities of animals, cross-diffusion of relatively low molecular substances, including crystalloids in body fluids and saline solution, takes place through the cellophane membrane; but higher molecular substances including protein of body fluid cannot diffuse through the cellophane membrane. Bacilli are cultivated in a medium consisting of low molecular substances and containing no protein.

By this cellophane chamber method it was found that avian bacilli grew, but that no growth of virulent bovine or human tubercle bacilli occurred in rabbits (figure 6). It is inferred, therefore, that the mechanism of the pathogenicity of avian tubercle bacilli in rabbits is not the same as that of the bovine and human-type tubercle bacilli.

In the next experiment, a cellophane chamber was made containing Kirchner's medium with 10 per cent rabbit's serum, and a small air bubble was allowed to remain in the chamber. This chamber is called cellophane chamber *K*. In this chamber *K*, bacilli, embedded in paraffin oil and separated completely from the outer world, can grow very well. Therefore, it is certain that in this chamber *K* conditions for bacillary cultivation are excellent.

Bacilli inoculated into chamber *K* were placed in the peritoneal cavity of animals and exposed to a medium containing 10 per cent rabbit's serum and low molecular substances of body fluid.

By this method the particular characteristics of smegma bacilli were determined. In chamber *O*, smegma bacilli could not proliferate in rabbits. In chamber *K*, the smegma bacilli proceeded to grow and after twenty days very large colonies developed (figure 7). From these experiments it was found that the growth-inhibiting factor of body fluid to the smegma bacilli is contained in the high molecular substances, perhaps in the protein fractions.

In summary: The mechanism of natural and acquired resistance of animals to tubercle bacilli has been investigated with particular reference to the role of the "humoral factor." For this purpose a new chamber method was developed. The chamber method is very convenient for observing and analyzing the growth-inhibiting or growth-promoting functions of body fluids on tubercle bacilli. With various modifications of the method, it is possible to observe the growth of several different strains of bacilli at the same time in the same peritoneal cavity of the same animal. The mechanics of the method are not very difficult or troublesome and, after a relatively short experience, it is possible to implant approximately sixty chambers into 10 rabbits within an eight-hour period.

The Tuberculosis Research Institute
Kyoto University
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Shusuke Tsuji
Kaoru Ito

January 19, 1955

Correspondence

THE PROBLEM OF ANTIMICROBIAL TREATMENT OF PRIMARY TUBERCULOUS INFECTION IN CHILDREN

To the Editor of The American Review of Tuberculosis and Pulmonary Diseases:

The introduction of specific drugs in the treatment of tuberculosis raises the important question of their use in cases of primary tuberculous infection. In many centers, children hospitalized for primary tuberculosis have been treated with these drugs and it is a common experience that they do not influence the usual course of this condition.

Clinically, the vast majority of children with primary tuberculosis are free from marked constitutional disturbance and it is difficult, therefore, to assess the influence of drugs on the basis of clinical findings. Roentgenographically, the shadows cast by the enlarged mediastinal lymph nodes, primary foci, or by the lobar and segmental lesions certainly do not melt away under the influence of the antituberculous drugs in current use. It is probably true to say that the shadows of these lesions behave in exactly the same way in untreated children as they do in those receiving intensive treatment. Bronchial obstruction due to tuberculous granulation tissue or lymph node pressure is not an uncommon finding in children with segmental lesions. Antimicrobial drugs do not apparently affect such endobronchial findings, as can readily be shown by repeated bronchoscopies of treated cases.

While the antituberculous drugs do not apparently influence the course of primary tuberculosis, it is hoped and commonly believed that their administration in cases of primary infection prevents, or at least diminishes, the incidence of complications and sequelae of this condition.

It is the *preventive* aspect of the treatment of primary tuberculosis, whether by antimicrobial drugs or by any other measures, that is important.

Further discussion will be limited to this aspect of the problem and will deal with all cases of recent primary infection, irrespective of the presence or the absence of the roentgenographic abnormality. Such apparent disregard for the roentgenographic abnormalities is justified by the findings of the High Wood study[1]. It has been shown there that the type and extent of roentgenographic abnormality on conversion has little bearing on prognosis.

Before proceeding with any further discussion on the subject of the treatment, it is essential to form as accurate a picture as possible of the complications we are trying to prevent, their relative importance, frequency, and time of onset. These can most conveniently be considered under three headings[2]:

I. *Benign pulmonary complications:*

a. Segmental lesions and

b. Pleural effusion

These are the most frequent complications of primary tuberculosis. The frequency with which they occur is approximately 10 per cent when both are taken together, the segmental lesions being more common in the younger, and pleural effusion in older, children.

Segmental lesions occur early, either at the time of tuberculin conversion or within a few months. The majority of the cases of pleural effusion arise a little later, between the second and sixth months.

[1] BENTLEY, F. J., *et al.*: Tuberculosis in Childhood and Adolescence, National Association for Prevention of Tuberculosis, London, 1954.

II. *Complications due to hematogenous dissemination:* Tuberculous meningitis, the most common cause of death in infected children, is the most important in this group, which also includes conditions such as miliary disease, skeletal tuberculosis, genitourinary tuberculosis, and a proportion of cases of cervical adenitis, et cetera. These complications occur much more frequently in infants than in older children. In the High Wood study their incidence in children less than two years of age was approximately 10 per cent, while in children more than two it was only about 3 per cent. Cammock and Miller[2] estimated that the risk of developing meningitis and miliary tuberculosis among infected children less than five years of age is 4.1 per cent. Holmdahl[3] found in his study of 657 children with primary tuberculosis and erythema nodosum that the risk of hematogenous complications was 6.2 per cent. It is conceivable that studies in the countries where malnutrition among children is common would reveal a higher incidence of hematogenous complications following primary infection.

The serious complications of this group, namely, tuberculous meningitis and miliary tuberculosis, tend to occur early in the course of primary tuberculosis, usually within the first six months following tuberculin conversion. Other complications such as skeletal tuberculosis are scattered over a wide period of time but most are found within the first few years, the blood-borne seed evidently taking longer to mature into an overt disease in the bones than in the nervous system.

III. *Chronic pulmonary tuberculosis*—adult or reinfection: This constitutes the other grave danger of primary infection. Its time relationship to tuberculin conversion is inconstant. In some cases chronic pulmonary tuberculosis follows primary infection almost immediately; in others, many years or even decades separate the two. This condition does not usually appear until adolescence. The scarcity of long follow-up studies and the absence of life-long investigation make it impossible to assess the frequency of this risk accurately. In the High Wood study with a seven-year observation period, we have assessed the risk of developing chronic pulmonary tuberculosis between twelve and twenty years of age to be about 5 per cent. Lincoln[4] found that 8 per cent of tuberculous children developed this type of disease one to fourteen years after primary tuberculosis had first been diagnosed. Holmdahl[3] found the total risk after twenty years to be 9.2 per cent. Nissen Meyer[5] found that the total morbidity from chronic pulmonary tuberculosis was approximately 3 per cent for those infected before the age of thirteen and more than 10 per cent for those infected after their thirteenth birthday. It should be stressed that the danger of developing chronic pulmonary tuberculosis is greater in older children and adolescents with primary infection than in those infected at an earlier age.

To summarize, a recently infected child may develop three main types of complications and sequelae: first, segmental lesions and pleural effusion; second, the complication due to hematogenous spread; and third, the grave but sometimes remote possibility of chronic pulmonary tuberculosis. It would appear from the High Wood study that 15 per cent of these infected children will develop some complication. The type will vary greatly with age; in children less than two years, 15 per cent will be accounted for chiefly by the hematogenous complications and, to a lesser extent, by segmental lesions. In children between two and twelve, the emphasis shifts to segmental lesions and pleural effusion. Last,

[2] Cammock, R. M., and Miller, F. J. W.: Lancet, 1953, *1*, 158.

[3] Holmdahl, K.: Acta tubere. Scandinav., 1950, supplement 22.

[4] Lincoln, E. M.: Am. J. Med., 1950, *9*, 623.

[5] Nissen Meyer, S.: Statistical Investigations of the Relationship of Tuberculous Morbidity and Mortality to Infection, E. Munksgaard, Copenhagen, 1949.

in older children, although the chief emphasis remains on pleural effusion, chronic pulmonary tuberculosis will become a relatively frequent danger.

There is no agreement on the subject of indications for antimicrobial drugs among various authorities. Some consider that it is better to replace active treatment by observation, in order to diagnose early and treat promptly any complications which might develop.[6] Others advocate thorough treatment for every recent tuberculin convertor, irrespective of age[7]. Others still treat only selected groups, e.g., infected infants[8] with bronchoscopic abnormalities[9].

There are three main considerations which must govern any discussion on this subject:

1. Do the dangers of primary infection warrant prolonged antimicrobial therapy?
2. Is such therapy effective?
3. Do the possible toxic effects and disadvantages preclude the use of antimicrobial treatment?

The present experience shows that antimicrobial treatment is not wholly effective and that some complications occur in spite of it. Thus, for instance, pleural effusion and segmental lesions can and do arise during the most intensive course of treatment. There are reasons to believe, however, that antimicrobial therapy will reduce the incidence of all complications and particularly the dreaded complications of hematogenous dissemination. Well-conducted therapeutic trials are necessary to assess just how effective such treatment is, and future policy must be primarily guided by results of these trials.

The serious toxic effects are uncommon; the mild ones, unimportant. We have no knowledge of any remote toxic effects, and such a theoretical possibility should not be given undue weight. The danger of producing bacterial resistance may well prove to be a real one, particularly when such treatment is carried out with one drug alone. It is safer at present to accept the principle that, if a certain combination of drugs is considered necessary for treatment of tuberculous disease, it should not be departed from to any great extent in the prophylactic treatment. The expense of the antimicrobial drugs is a major consideration in many instances.

Finally, consideration must be given to the adverse psychological effect of treatment in a stage of the disease which until recently was regarded in many parts of the world as a normal biologic phenomenon. This effect would, of course, be markedly increased if such individuals were required to submit to sanatorium treatment. Antimicrobial treatment of primary infection should be ambulatory, and only very few restrictions need be enforced on the ordinary life of infected children.

We can only hope to define the proper indications for the antimocrobial therapy of primary tuberculosis when the results of the therapeutic trials are known. In the meantime, in the writer's opinion, treatment should be limited to the following groups of children:

1. Recently infected children less than two and more than twelve years of age.
2. Selected groups of recently infected children between these ages.

The following groups should be treated: (*a*) those with a severe degree of pyrexia and constitutional disturbance lasting for more than one month following tuberculin conversion; (*b*) those with a bad family history and particularly those giving a history of a recent death from tuberculosis among members of the immediate family; and (*c*) those showing definite enlargement of paratracheal lymph nodes in the roentgenogram.

[6] Editorial: Primary tuberculosis in children, New England J. Med., 1954, *251*, 716.

[7] Waring, J. J.: Dis. of Chest, 1954, *25*, 361.

[8] Wallgren, A.: Tuberculosis and Other Problems of Pediatrics, Williams and Wilkins, Baltimore.

[9] Myers, J. A.: Dis. of Chest, 1952, *22*, 293.

It will be appreciated that the occurrence of tuberculous complication constitutes in most instances an indication for antimicrobial therapy; but this subject is beyond the scope of the present letter.

3. Children of any age hospitalized for primary tuberculosis.

The aims of institutional treatment of primary tuberculosis are the same as those of antimicrobial therapy, i.e., primarily the prevention of complications and sequelae. The value of this treatment remains controversial and has been discussed elsewhere[9].

There are good reasons to hope that antimicrobial therapy will prove a much more potent weapon in our hands than the treatment by bed rest and sanatorium routine. It is surely illogical to use the more costly and less effective weapon of hospitalization and at the same time withhold the antimicrobial therapy.

* * *

It is not necessary to bring forth any further arguments for the desirability of the therapeutic trials on large groups of children with primary infection, particularly as such trials are already being undertaken.[10] It can confidently be hoped that such large-scale studies will not only provide an answer to the question of the efficacy of antimicrobial treatment, but that they will also fill many gaps in our knowledge of tuberculosis.

The random allocation of cases to the treated and untreated groups will ensure that they are comparable with respect to age and the duration of interval between infection and commencement of therapy.

Diminution in the number of all or any of the complications will be the most important measure of efficacy of the drugs. The up-to-date experience with antimicrobial agents does not suggest that much will be gained from the study of temperature, erythyrocyte sedimentation rate, and weight charts, or even from the assessement of roentgenographic progress of primary lesions. In these respects such trials will differ from those carried out on adults with chronic pulmonary tuberculosis. As most of the complications occur early in the primary disease, fairly conclusive results of the trials may be expected within a short time.

No discussion on treatment of primary tuberculous infection would be complete without some consideration of case finding. The dangers attending primary infection are sufficiently high to warrant a determined effort to detect recently infected children, whether for the purpose of antimicrobial treatment or to ensure thorough supervision and early diagnosis of complications. At present the vast majority of such children remain unknown. We have shown in the High Wood study that the two common ways in which children with primary tuberculosis are diagnosed are as a result of either contact examination or investigation of more severe examples of the "primary illness."[11]

The only effective method for the diagnosis of all infected children is by periodic tuberculin testing. A reliable tuberculin test must be used, and examination must be repeated at frequent intervals if it is to lead to discovery of really recent convertors and thus cover the most dangerous first months after infection. Yearly intervals between tests is too long, as cases then discovered would include children infected many months previously who have already passed through the most dangerous period; yet more frequent testing may prove unrewarding in communities with a low conversion rate. If yearly testing is decided upon, advantage should be taken of the fact that in temperate

[10] Lincoln, E. M.: Am. Rev. Tubere., 1954, *69*, 682.

[11] Wallgren, A. and Miller, J. A.: Pulmonary Tuberculosis in Adults and Children: Part II, Thomas Nelson, New York, 1939.

climates most primary infections occur during the cold season and, therefore, the optimal time for tests is in the spring.

In summary, it may be said that the most important aim of antimicrobial treatment is prevention of complications and sequelae of primary infection. The dangers of primary tuberculosis are discussed in relation to their frequency, seriousness, and time of onset. They are:

a. Early, frequent pulmonary complications: segmental lesions and pleural effusion.

b. Complications due to hematogenous dissemination. The most serious of those, meningitis and miliary disease, occur early, and they are more frequent in infancy than later.

c. Chronic pulmonary tuberculosis arising more often in older children and adolescents.

Present experience indicates that antimicrobial treatment will not prevent all complications. Large-scale trials are necessary to decide on the degree of efficacy of the treatment.

Antimicrobial treatment does not seem to influence the clinical and roentgenographic course of primary tuberculosis.

Antimicrobial treatment of primary infection should be ambulatory.

Pending the results of therapeutic trials, treatment should probably be limited to the following groups of recently infected children:

a. Those less than two and more than twelve years of age

b. Those with severe prolonged illness, with very bad family history, and with enlargement of paratracheal glands

c. Those hospitalized for primary tuberculosis

A determined effort should be made to diagnose all recently infected children by periodic tuberculin testing.

Division of Tuberculosis Prevention
Department of Health
Parliament Buildings
Toronto 2, Ontario

Sincerely yours,
Stefan Grzybowski

April 13, 1955

Books

THE BROMPTON HOSPITAL. By Maurice Davidson and R. G. Rouvray. Pp. 151, Lloyd Luke, Ltd., London, 1954.

This history of the "Brompton Hospital for Consumption and Diseases of the Chest" in London, recorded for the first time, should be of general interest to laymen and practitioners alike, but to those who have been connected with the Brompton it should give particular pleasure.

This is an excellent production, well illustrated, with writing which clearly betrays the style of Dr. Maurice Davidson, at present a senior member of the consulting staff. Together with Mr. Rouvray, for many years House Governor, Dr. Davidson traces the history of the Hospital, its staff, and administration from their modest beginnings in the Manor House, Chelsea, in 1842 (where 20 patients were accommodated) to the present time when more than 1,800 patients are admitted and more than 70,000 attendances are recorded in the outpatient department each year.

Of particular interest are the illuminating sketches of erstwhile physicians and surgeons on the staff, including such names as Quain, Ewart, Lister, and Fowler; also the part played by the Brompton in the Sanatorium Movement when, in 1853, a sanatorium was opened in Bournemouth for the reception of selected patients who might benefit from a congenial climate.

The Hospital owes its foundation to one Philip Rose who recognized the plight of victims of tuberculosis who were outcast from society and institutional care. It becomes evident just how much imagination, energy, and tenacity were needed to persuade his contemporaries to support this venture at a time when it was recorded in an appeal for funds: "Consumption the most frequent and fatal disease in existence which according to high authority, destroys one third of our entire population, finds the doors of all our hospitals closed against it."

From that time on, the gradual growth of the Hospital is described during more than a hundred years of great change in social structure, attitudes to disease, and the practice of medicine. At present, in the setting of the National Health Service, the Brompton maintains its identity and autonomy as a great institution and the foremost center of thoracic medicine in the United Kingdom.

JOHN BATTEN
London, England

PERSPECTIVES AND HORIZONS IN MICROBIOLOGY. A symposium. Edited by Selman A. Waksman, with 16 contributors. Pp 220. Rutgers University Press, New Brunswick, N. J., 1955. Price, $3.50.

This excellent symposium on microbiology was arranged in connection with the dedication of the Rutgers University Institute of Microbiology on June 7, 1954. The University and Dr. Waksman are to be congratulated for bringing together this outstanding group of scientists whose presentations helped to make the dedication of the new Institute a milestone in the history of microbiology.

The thirteen chapters, each written by a different authority, are divided into three parts, entitled: "The Microbe as a Living System," "Metabolism of Microorganisms," and "Microorganisms and Higher Forms of Life." An appendix contains the dedication addresses of Lewis Webster Jones, President of Rutgers University; Dr. Selman A. Waksman; and Dr. Albert J. Kluyver.

Within these thirteen chapters one gets a panoramic view of the present and future aspects of a "science devoted to the study of the microscopic forms of life and their relation to mankind." One sees how the fields of bacteriology, biochemistry, genetics, mycology, enzymology, immunology, virology, and plant biology are intermingled in the broad science of microbiology. Stressing mainly its purely scientific aspects, a few papers in the symposium nevertheless indicate some of the practical applications, such as the production of antibiotics and the fascinating way in which microorganisms have helped to solve complex problems in steroid organic chemistry. Many interesting historic facts are also brought out, including the observation in 1891 that certain cases of arsenic poisoning were related to the liberation of volatile compounds originating from arsenic-containing pigments by fungi growing on wallpaper.

Although some of the chapters are highly technical, the text in general is well integrated and certainly worth the time of anyone interested in this burgeoning branch of science.

EMANUEL WOLINSKY
Trudeau, New York

A PRACTICAL MANUAL OF DISEASES OF THE CHEST. By Maurice Davidson. Pp. 647, Oxford University Press, London, 1954.

With his fourth edition of this book Maurice Davidson has added to the recent texts of pulmonary disease one notable for both its readability and its obvious basis upon long clinical experience and patient contact.

The organization of this manual is conventional, the format pleasant, the style tending toward the informal and unusually easy to read. The appropriate use of case material and excellent choice of representative roentgenograms and diagrams add considerably to the over-all value.

In common with most other modern medical texts, however, this too suffers from the rapid evolution of therapeutic concepts and details which outstrip all attempts at revision of standard texts. Thus the chemotherapy of tuberculosis, of the bacterial pneumonias, and of chronic suppurative lung disease, for example, remains in the realm of the journals. The effects of steroid therapy and the usefulness of exfoliative cytology are scarcely touched upon. One regrets even more the lack of a section dealing with any of the newer concepts of pulmonary physiology, and the chapter on oxygen therapy would benefit by expansion.

The American reader will perhaps wish for a more detailed bibliography of the United States literature and will find the details of therapy somewhat foreign in tone and nomenclature, although the former can be obtained from any equivalent U. S. text and the latter is of considerable casual interest.

In the preface to the first edition of the book, Dr. Davidson states its goal as one of filling a need somewhere between the exhaustive treatise and the brief manual of chest disease. His clinical descriptions, his observations on practical management, and the section on physical diagnosis of the chest are of particular merit in achieving his end and offer both profit and pleasure. For recent developments in theory and therapy, for precise or elaborate detail, and for rare chest diseases or pulmonary manifestations of essentially nonpulmonary diseases, one must search elsewhere.

BARBARA BATES
New York, New York

MANUAL OF CLINICAL MYCOLOGY (second edition). By Norman F. Conant, David Tillerson Smith, Roger Denio Baker, Jasper Lamar Calloway, and Donald Stover Martin. Pp. 456, W. B. Saunders Co., Philadelphia, Pa. 1954. Price, $6.50.

The first edition of this book, published in 1944 as part of the Military Medical Manuals program of the National Research Council, has been extensively revised and expanded. As the authors' special fields encompass mycology, bacteriology, pathology, and dermatology, this book is evidently an attempt to correlate the laboratory aspects of mycology with the clinical management of the fungous diseases. The mycoses are discussed from the viewpoint of definition, geographic distribution, incidence, symptomatology, mycology, pathology, immunology, differential diagnosis, prognosis, and treatment. The mycologic and immunologic aspects are thoroughly covered. Clinical differential diagnosis and treatment are less adequately covered, particularly in the sections dealing with superficial fungous diseases. However, advances in therapy, such as use of stilbamidine in the treatment of blastomycosis, are included. The text is well illustrated and a representative up-to-date bibliography is appended to each chapter.

The major portion of the book deals with the systemic mycoses such as actinomycosis, blastomycosis, coccidioidomycosis, sporotrichosis, et cetera. In those mycoses with pulmonary involvement, reproduction of roentgenograms showing involvement are used in illustration. Chapters on "Fundamentals of Medical Mycology" and "Contaminants" and an appendix discussing mycologic, pathologic, immunologic, and dermatologic methods are included.

This manual should prove most valuable to mycologists, immunologists, pathologists, dermatologists, and specialists in tropical diseases. Physicians interested in chest diseases will find the chapters on the systemic mycoses useful. The term "clinical" in the title may be somewhat misleading to general practitioners interested chiefly in the clinical differential diagnosis and management of the more common superficial fungous diseases.

DOUGLAS TORRE
New York, New York

TUBERCULOSIS IN IRELAND. Report of the National Tuberculosis Survey (1950–1953). Pp. 301, Medical Research Council of Ireland, Dublin, 1954.

This report of the National Tuberculosis Survey of the Republic of Ireland embodies the results of a study of the over-all problem of tuberculosis in that country. It is timely in that it appears following a concerted attack on this problem and, therefore, defines the areas toward which more concentrated efforts can be made. The most important details of the report are the assessment of methods of case finding, a study of the progress of the disease in a group of early cases, and the factors favoring its spread. This includes an evaluation of group susceptibility, the influence of social and economic factors, and the extent of infection throughout the population. The report ends with a forthright criticism of the services provided to date, comparison with the methods employed and results obtained in other European countries, with thoughtful recommendations for improvement.

Mortality figures, comparing the age groups, show that a peak occurs at lower ages than in other countries and this, together with the fact that a large percentage of patients have far advanced tuberculosis when first diagnosed, constitute findings which are of considerable significance. This is reflected in the large proportion of patients subjected to pneumonectomy and lobectomy and the relatively small number of segmental resections performed.

Tuberculin testing in rural areas tends to indicate the small part played by bovine infection, and figures for a small series of strains studied suggest an incidence of bovine tuberculosis which closely parallels that of England and Wales, though considerably higher than that of Northern Ireland and lower than that of Scotland. The low prevalence of tuberculin reactors in rural areas, from which the greater number of emigrants to other countries is drawn, immediately suggests the role played by the urbanization of tuberculin-negative susceptibles, for in this population the morbidity rate is high. Thoughtful consideration is given to the possibility of offering BCG vaccination to all nonreactors prior to emigration.

Mass roentgenographic surveys indicate a higher incidence of active disease than in most European countries, though lower than in certain others, but the usual difficulties were experienced in obtaining an adequate response and follow-up. That surveys probably miss a proportion of persons with active disease, particularly in the older age group, is indicated by the continued morbidity rate, compared with the relatively small number of patients remaining in their homes and who are known to have active disease.

As is happening elsewhere, modern methods of treatment have greatly reduced the mortality figures, but the morbidity rate for the disease remains high. This may indicate dissemination of infection by patients who have survived in spite of extensive disease, or it may be that modern treatment methods have simply outreached methods of prevention and case finding. The latter, as is common elsewhere, would appear to be the weakest aspect of the campaign and one which has received due consideration in attempts to institute treatment earlier and at a time when extensive and irreversible damage has not already taken place. From the figures available, it would appear that the unknown cases constitute a greater hazard in relation to the continued high morbidity, but the proportion of previously treated cases responsible in part can only be determined by extensive bacteriologic study of known cases.

This report represents a carefully planned and coordinated investigation into all possible aspects of the problem, many of which have not of necessity been fully evaluated, owing to the lack of adequate study material. It will be of great interest to all those involved in the prevention and treatment of tuberculosis. The material, though disappointing in some of its aspects through its lack of detail, is well presented. It is an honest appraisal of the situation and aptly fulfills the purpose for which it was intended.

David G. Simpson
New York, New York

BOOKS RECEIVED

The Biologic Effects of Tobacco. Edited by Ernest L. Wynder, Pp. 215, Little Brown and Company, Boston and Toronto, 1955, Price, $4.50.

Tuberculosis and Aspiration Liver Biopsy. By A. J. Ch. Haex and Cornelia Van Beek. Pp. 100, De Erven F. Bohn N.V., Haarlem, Holland, 1955.

Annual Review of Medicine. Volume 6, Pp. 459. Annual Reviews, Inc., Stanford, California, 1955, Price, $7.00.

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American Trudeau Society

Medical Section of the National Tuberculosis Association

Antimicrobial Therapy of Tuberculosis

A Statement of the Committee on Therapy

Indications

Antimicrobial therapy has become established as the keystone in the medical management of tuberculosis. There is agreement among authorities that it is indicated in all instances of active tuberculosis of any organ or system of the body. There are virtually no exceptions to this general statement of indications except with respect to the problem of recent infection which is manifested only by a skin reaction to tuberculin. It has not been established that persons with no other evidence of tuberculosis than a tuberculin skin reaction require treatment or that antimicrobial therapy is of preventive value in this situation, even when the onset of tuberculin sensitivity is recent. Current controlled studies of the treatment of recent reactors in various age groups will require several years at least for evaluation. Meanwhile, since the risk of meningitis is high in infancy and since there is evidence that this particularly dangerous form of the disease may be prevented by isoniazid, recent infection in this age group, even though manifested only by the tuberculin skin test, may reasonably be treated prophylactically.

Available Drugs

Several agents are officially approved and available for general use in the treatment of tuberculous disease. Of these, *isoniazid* and *streptomycin* are the most effective. They are administered with each other, or one or both with *para-aminosalicylic acid* (PAS). While there is general agreement that neither streptomycin nor PAS should be administered alone, there is disagreement whether isoniazid may be administered alone in certain clinical situations. There is as yet no clear agreement with respect to which of the possible combinations of these three drugs may be the antimicrobial "therapy of choice" in the initial treatment of patients never previously treated with any of these agents.

In addition to the three most commonly used antimicrobial agents, there are several others, among the many drugs which have received extensive clinical trial, which are approved or are regarded as still promising. Most important among these are *viomycin*, which is approved and available for general use, and *pyrazinamide*, which is currently under extensive clinical study but has not been approved for general use.

The role of viomycin is secondary, and its usefulness appears to be restricted to situations in which neither isoniazid nor streptomycin can be used to advantage, either because of intolerance to these agents or high degrees of bacterial resistance. Pyrazinamide may prove to be of value, particularly as a companion drug to isoniazid in initial treatment. Its usefulness otherwise has been found to be limited because of its short-term efficacy when administered alone. The principal limitation in the use of pyrazinamide, however, results from its potential hepatic toxicity. The incidence of serious hepatic toxicity, at the dosage level which has been shown in earlier trials to be therapeutically effective, is apparently sufficiently great to preclude at the present time the general use of this drug. *Oxytetracycline* has been reported to have an effect similar to that of PAS in prolonging the therapeutic efficacy of streptomycin when it is administered

concurrently with this drug. It has not been used extensively with isoniazid, PAS, or other drugs.

Standard Regimens

Disregarding the less effective or more dangerous drugs, except as possible future additions or for rare special indications, there are, nevertheless, a number of effective regimens involving only the three more important drugs from which the clinician may make his choice. Those may be listed as follows:[1]

1. Streptomycin-PAS: 1.0 gm. of streptomycin intramuscularly two or three times weekly and 12 gm. of PAS (or 15 gm. of sodium PAS) daily by mouth in three or four divided doses
2. Isoniazid alone: 5 mg. or more of isoniazid per kg. of body weight per day in two or three divided doses
3. Isoniazid-streptomycin (both daily): 5 mg. or more of isoniazid per kg. and 1.0 gm. of streptomycin intramuscularly
4. Isoniazid daily—streptomycin twice weekly: Same dosage as in regimen 3, but streptomycin given twice weekly instead of daily
5. Isoniazid-PAS: 5 mg. or more of isoniazid per kg. per day and 12 gm. of PAS daily
6. Isoniazid-streptomycin-PAS: each in dosage as above; streptomycin given daily or twice weekly

Selection of Regimen

Regimens 1 and 2, streptomycin-PAS and isoniazid alone, appear to be somewhat less effective than the other regimens, as judged by organized cooperative trials in *pulmonary* tuberculosis. Percentage advantages in favor of regimens 3 to 6, in terms of such clinical criteria as roentgenographic improvement and sputum culture "conversion," are consistently found in various reports of large-scale comparative regimen trials. These advantages, however, are not large and other factors may be considered in selecting a regimen for a given patient with pulmonary and/or extrapulmonary tuberculosis.

Until very recently, and largely because of longer and more extensive experience, streptomycin-PAS was most often recommended as the therapy of choice for initial

[1] The dosages of the various drugs here indicated are for all forms of tuberculosis except the miliary and meningeal. In the latter, isoniazid is recommended in the dosage of not less than 8 to 10 mg. per kg. of body weight per day for the first two or three weeks, after which it may, except in unusual circumstances, be reduced to the 5 mg. level. In these forms it is also recommended that streptomycin be administered daily, rather than twice weekly, at least during the first three months of concurrent administration with isoniazid, and regardless of whether or not PAS is included as a third drug. The recommended regimen for miliary and meningeal tuberculosis is thus different from any of the standard regimens here listed and consists of 8 to 10 mg. of isoniazid per kg. per day in the first two to three weeks, then 5 mg. per kg. per day (Dosages of isoniazid in adults up to 16 mg. per kg. per day are now under investigation. Concurrent administration of vitamin B complex, with especial emphasis on pyridoxine, is indicated to prevent neuritis when high dosages are used.); 1.0 gm. of streptomycin daily for at least three months, then twice or thrice weekly if progress has been satisfactory; 12 to 20 gm. of PAS per day (regarded as optional by a minority of the committee).

For more detailed information concerning the treatment of miliary and meningeal tuberculosis, *see* "A Statement by the Committee on Therapy Concerning the Treatment of Tuberculous Meningitis" (Am. Rev. Tuberc., 1954, *70*, 756.)

therapy *in pulmonary*[2] and in most extrapulmonary forms of tuberculosis. This committee no longer considers this a valid recommendation. There is agreement that isoniazid *must be included* in the treatment of the most immediately dangerous forms of tuberculosis, namely, miliary, meningeal, and acute extensive pneumonic pulmonary tuberculosis. In less immediately life-threatening forms of tuberculosis, there is more latitude of choice. Streptomycin-PAS is still considered to be among the acceptable regimens, although many favor the inclusion of isoniazid in any regimen for initial treatment.

Multiple-drug regimens, including isoniazid, are thus most favored, but the choice among these on present evidence is a difficult one. Regimen 6, isoniazid-streptomycin-PAS, has not been demonstrated to have any clinical advantage over regimens 3, 4, and 5, the two-drug regimens containing isoniazid. Intolerance to one or more of the drugs, furthermore, is encountered most frequently in the three-drug regimen. Bacteriologic results, although they might reasonably have been expected to be superior, have not in fact been shown to be so with the three-drug regimen in pulmonary tuberculosis. However, in genitourinary tuberculosis the three-drug regimen appears to have a definite advantage in the bacteriologic results. The choice of a multiple-drug regimen for pulmonary tuberculosis which is both the most *effective* and *least toxic* appears on present evidence to lie between isoniazid-streptomycin and isoniazid-PAS. There is no clear-cut advantage in one of these two-drug combinations over the other. Isoniazid-streptomycin with streptomycin given daily is probably more effective than when streptomycin is administered only twice weekly.[3] If isoniazid-streptomycin is selected it may prove to be advisable, therefore, to give the streptomycin daily, at least during the first two or three months of therapy.

Isoniazid, as a single-drug therapy, is little favored in the published literature. This regimen was dropped early in most of the large comparative regimen trials, mainly because the *in vitro* tests suggested a probable early loss of effectiveness due to drug resistance. Good results in miliary tuberculosis, however, and the limited evidence available with pulmonary tuberculosis indicate that the correlations between *in vitro* drug resistance and *in vivo* response to treatment are less clear-cut than they are with streptomycin. Clinical results have been much more favorable with isoniazid than with any other antituberculous drug when given alone. Like streptomycin-PAS, isoniazid alone cannot be classed as a maximally effective regimen, but its ease of administration and relatively low toxicity compel a consideration of its use. The precise indications in which isoniazid alone may prove to be the most desirable initial therapy are not yet defined. Its possible field of usefulness in certain situations in which circumstances make the simplest therapy especially desirable are in need of exploration and systematic study. Such special situations include: (*1*) treatment of the recent tuberculin reactor; (*2*) outpatient treatment in the various phases of management of the unhospitalized patient, including the post-hospital continuation therapy of patients treated successfully with other regimens; (*3*) prophylactic therapy to "cover" steroid hormone administration in chronic nontuberculous diseases; (*4*) noncavitary pulmonary tuberculosis; and (*5*) various closed forms of extrapulmonary tuberculosis.

Duration of Antimicrobial Therapy

Regardless of which of the regimens is selected for initial therapy, there is agreement and overwhelming evidence that *continuous* antimicrobial therapy should be maintained

[2] Am. Rev. Tubere., 1951, *63*, 617.

[3] British Medical Research Council: Various combinations of isoniazid with streptomycin or PAS in the treatment of pulmonary tuberculosis (7th Report), Brit. M. J., February, 1955.

for long periods of at least twelve months, and most often for eighteen months or even longer. When serious intolerance or toxicity develops to one drug or pair of drugs, other antimicrobial therapy should be substituted.

Major Toxic Effects

The major toxic effects of streptomycin and of isoniazid are well known and can usually be avoided by not exceeding the recommended doses. Certain precautions are, however, to be emphasized. In the presence of renal insufficiency, streptomycin in any dosage becomes dangerous and should be avoided. Since it is itself nephrotoxic, and the concentration in the blood is elevated when renal function is impaired, it should usually be discontinued on the first appearance of any significant degree of renal excretory impairment. The peripheral neuritis sometimes caused by isoniazid may occur more easily under circumstances of renal impairment, alcoholism, and other deficiency states. It occasionally occurs in the absence of these circumstances and at the recommended dosage level. Careful examination will reveal subclinical cases without spontaneous complaints. Central nervous system effects of isoniazid consist mainly of the precipitation of convulsions or of psychoses in persons predisposed or with past histories of such disorders. Although not necessarily contraindicated, because even in these circumstances the precipitation of attacks is rare, the drug should be discontinued immediately on the first indication of such effects. Hypersensitivity reactions to each of the three drugs are encountered and these always require immediate interruption. PAS is the most frequent offender in this regard. Hypersensitivity to more than one drug may appear simultaneously. In such circumstances, desensitization may be corrective. Steroid hormone therapy has also been used successfully in overcoming drug hypersensitivity.

Dihydrostreptomycin may be substituted temporarily when necessary for streptomycin, but its long-term use should be avoided when possible because of the danger of auditory toxicity. Viomycin should probably not be used concurrently with streptomycin, since their neuro- and nephrotoxic effects are qualitatively similar and may be additive.

Bacterial Susceptibility

Discontinuance of either streptomycin or isoniazid, and substitution of the other, may be advisable when the clinical progress has clearly ceased to be favorable. Especially is this true if *in vitro* tests of bacterial susceptibility show a preponderance of resistant organisms. In the case of streptomycin, an *in vitro* inhibitory concentration above 25 γ per ml. may be regarded as almost certain evidence of loss of *in vivo* activity, especially if this is found in more than one culture. Levels of *in vitro* resistance between 10 γ and 25 γ are indicative of doubtful therapeutic effect; susceptibility to 1 γ or less is the only sure sign of maximal antimicrobial effect. In the case of isoniazid, the correlation between *in vitro* susceptibility tests and therapeutic effect is less definite. Although resistance to 5 γ per ml., or even to 1 γ, has been widely regarded as indicative of loss of antimicrobial effect, this is not firmly established. Even resistance to 20 or 25 γ per ml., as demonstrated by the conventional methods, is not always clinically significant. This probably arises from the fact that the conventional tests do not measure the actual proportion of resistant cells in the culture which may be demonstrated even though present in relatively small numbers. PAS-susceptibility tests are unfortunately rarely performed. Failure *in vivo* coincides with *in vitro* resistance to 5 γ or more per ml. There is evidence that, when resistance to PAS has developed, continued concurrent administration of this drug no longer reliably exerts a delaying action on the development of streptomycin or isoniazid resistance. In the case of viomycin, failure *in vivo* correlates fairly well with resistance to 25 γ per ml. Resistance tests with pyrazinamide are unreliable.

Inasmuch as susceptibility tests require three to eight weeks or more for their completion, they are of limited practical value as guides to therapy. *In vivo* response to the drug does not always parallel the *in vitro* findings, and the latter are not always consistent. Susceptibility tests should, therefore, be performed whenever possible, but should be evaluated in conjunction with clinical data. Initial therapy should usually not be delayed while awaiting results of these tests, nor should alterations in therapy be hastily made on the basis of these results alone.

Correlation with General Plan of Management

With the several effective drug regimens now available for the antimicrobial therapy of tuberculosis, it is no longer considered necessary to delay initiation of active treatment until hospitalization can be effected. Initiation of such treatment in the hospital is still considered preferable whenever possible.

Selection of an advantageous initial regimen may be made in any given case from the several regimens which have been listed and discussed. In making this selection, not only the type and severity of tuberculous disease, but the circumstances under which treatment must be carried out, may properly be considered. The multiplicity of choices does not imply a haphazard plan of treatment for best results. An over-all plan should always be formulated at the outset of treatment so that antimicrobial therapy may be correlated with the other modalities such as bed rest, collapse therapy, and surgery. Progress must be reviewed at frequent intervals and timely additions or changes made within the framework of the over-all plan of management.

Carl Muschenheim
For the Committee on Therapy

William Barclay
Paul Chapman
Raymond Corpe
Carl Muschenheim
Cecil G. Shaver
Herbert E. Sloane, Jr.
Kenneth M. Smith
Morris Thomas
Robert L. Yeager
Roger S. Mitchell, *Chairman*

The Chemotherapy of Tuberculosis in Childhood

A Statement of the Committee on Therapy

The chemotherapy of tuberculosis in infants and preadolescents is discussed under a suggested new classification of five categories:

1. *Miliary and meningeal tuberculosis:* Miliary tuberculosis, once almost invariably fatal, responded well to streptomycin and para-aminosalicylic acid (PAS), although some patients developed tuberculous meningitis while under treatment. Following the addition of isoniazid, meningitis has ceased to be a complication. Streptomycin and PAS caused a spectacular reduction in the mortality of tuberculous meningitis. Results have been significantly further improved by the addition of isoniazid. Daily isoniazid and streptomycin are indicated in all such cases. The majority of the committee consider that daily PAS is also indicated; the minority, that its use in such cases is optional.[4]

2. *Clinically active pulmonary and/or extrapulmonary tuberculosis:* All such cases require the prompt institution of chemotherapy, including isoniazid, to prevent progression and miliary or meningeal dissemination.

3. *Tuberculin reactors* (with noncalcific roentgenographic pulmonary or hilar lymph

[4] For more detailed recommendations, see the statement of this Committee on miliary and meningeal tuberculosis, Am. Rev. Tubere., 1954, *70*, 756.

node lesions, without symptoms and with negative bacteriologic findings): Despite the fact that most such lesions have healed spontaneously, too many have shown progression to the more dangerous forms of the disease. Careful evaluation of each case is indicated. In the absence of overt evidence of active, progressive or complicated disease, the use of chemotherapy is now under investigation. When the decision to treat has been made in the individual case, isoniazid in dosage of 5 mg.[5] per kg. per day for at least one year with or without PAS is the chemotherapy of choice.

4. *Tuberculin reactors* (with no demonstrable lesions in the lungs or elsewhere):
   a) Recent "conversion" (within one year). An increasing number of authorities are considering the advisability of prophylactic chemotherapy of patients in this category. The Committee is not prepared to make a recommendation at this time pending the outcome of controlled studies now in progress.
   b) "Conversion" date unknown. Such patients should be kept under observation for one year. Chemotherapy is not recommended unless more specific indications become manifest.

5. *Tuberculin reactors* (with inert calcific hilar or pulmonary shadows): While no treatment is indicated, such persons should be kept under observation.

In general, a routine tuberculin test using intermediate strength 0.0001 or 0.0002 mg. of PPD intracutaneously, or its equivalent, is recommended before one year of age and, if negative, should be repeated annually thereafter. The discovery of unsuspected cases by this technique leads to unsuspected sources of infection. The treatment of infected children should include separation from known sources of further infection. Lastly, it must be stressed that, the younger the child, the greater the risk of tuberculosis morbidity and mortality and, hence, the need for clearly effective treatment.

Kenneth M. Smith
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Genitourinary Tuberculosis

A Statement of the Committee on Therapy

Tuberculosis of the genitourinary tract should be sought in every diagnosed case of tuberculous disease, particularly the pulmonary, osseous, and miliary forms. Pyuria may appear many (up to twenty) years after pulmonary tuberculous infection.

Tuberculosis of the genitourinary tract is commonly encountered without demonstrable evidence of active tuberculosis elsewhere, the lesion which was the portal of entry having become quiescent meanwhile.

When tuberculosis of the male genital tract has been diagnosed, the urinary tract should be thoroughly investigated as the probable source of the genital infection.

The diagnosis should be established by guinea pig inoculation or by culture, whenever possible, before treatment with antimicrobial drugs is started. The presence of acid-fast bacilli on microscopy or suggestive roentgenographic findings are not usually sufficient

[5] Dosages in children up to 35 mg. per kg. per day are now under investigation. Concurrent administration of vitamin B complex, with special emphasis on pyridoxine, is indicated to prevent neuritis when high dosages are used.

grounds for initiating treatment, although a combination of these two findings may provide a satisfactory basis. Nephrectomy or epididymectomy for tuberculosis is never an emergency procedure and can wait, pending the results of culture or guinea pig examination. It is good practice to collect three twenty-four-hour voided urine samples in sterilized bottles by "clean-voided" technique. These should be kept cool and sent as quickly as possible for concentration and culture or guinea pig inoculation. This will reduce the number of specimens lost through contamination. The guinea pig method is usually superior to the culture method. If both examinations are performed on the same specimen, a higher percentage of positive results will be obtained than with either method separately. Catheter specimens from each ureter should be collected at cystoscopic examination, and should be submitted for both culture and guinea pig examination whenever practical. Drug-susceptibility tests should be performed on positive specimens.

Treatment Regimens

The treatment of genitourinary tuberculosis should follow the general principles of treatment for tuberculosis. Hospital care is indicated for investigation and initiation of treatment and, unless home conditions are excellent, the main course of treatment should be carried out in a hospital. Four chemotherapeutic regimens are now acceptable.

1. Streptomycin (1 gm. twice weekly) plus isoniazid (5 mg. or more per kg. per day) plus PAS (12 gm. daily) for one continuous year or more.

This "triple-drug" regimen appears to be slightly more effective than any combinations of two drugs yet tried, i.e., streptomycin-PAS and streptomycin-isoniazid, in the treatment of advanced renal or genital lesions. This is now the regimen of choice for genitourinary tuberculosis. It should be employed particularly for patients who have failed on other regimens. For patients who are inoperable, this regimen can be continued indefinitely or until toxic reactions force the discontinuance of one or more of the drugs.

2. Streptomycin (1 gm. twice weekly) and PAS (12 gm. daily) for one continuous year or more.

This regimen will usually arrest minimal renal lesions which show no deformity by pyelogram. It will also arrest some 70 per cent of advanced (cavitary) renal and genital lesions. It is useful in preparing patients for nephrectomy or epididymectomy, where the chemotherapy is directed in part against the subclinical tuberculous lesions which may exist in the contralateral "good" kidney.

3. Streptomycin (1 gm. twice weekly) plus isoniazid (5 mg. or more per kg. per day) for one continuous year or more.

This regimen appears to offer little or no advantage over streptomycin-PAS and has the disadvantage of committing the two most effective drugs.

4. PAS (12 gm. per day) plus isoniazid (5 mg.[6] per kg. per day) for one continuous year or more.

The effectiveness of this regimen is still under investigation and it may well prove to be as effective as triple-drug treatment for genitourinary tuberculosis.

The selection of a regimen for a patient with genitourinary lesions should always allow for the possibility that coexisting pulmonary or other tuberculous lesions might require a different combination of drugs for the particular situation. It is frequently necessary to defer to the requirements of the extragenitourinary lesions since pulmonary, miliary, or meningeal lesions may be more immediately dangerous to life.

[6] Dosages of isoniazid of up to 16 mg. per kg. per day are now under investigation. Concurrent administration of vitamin B complex with special emphasis on pyridoxine is indicated to prevent neuritis when high dosage is used.

General Therapeutic Considerations

a) In those cases in which *M. tuberculosis* is found in urine on culture, but show no evidence of abnormality in the pyelogram, good results can be expected by prolonged (twelve to eighteen months) chemotherapy, without resort to surgery.

b) In the event that a patient has a cavity in only one section of one kidney, it may be feasible to do a segmental resection of that kidney after four months of chemotherapy. Chemotherapy should then be carried on for a minimum of eight more months.

c) When there is one destroyed kidney, it is advisable to give a preliminary course of chemotherapy for a period of three weeks to four months before surgical excision of the destroyed kidney is considered. At nephrectomy, good results have been obtained in the disposition of the ureteral stump by bringing it to the skin at the anterior end of the nephrectomy wound. This technique makes it unnecessary to remove the ureter *in toto*.

d) Patients with bilateral renal tuberculosis, with cavity, on whom no thought of surgical excision can be entertained, should be treated with chemotherapy for a period of no less than one year and usually much longer.

e) To guard against the development of ureteral strictures, excretory urograms should be performed every four months throughout the treatment period. Cystoscopic dilatation of any such strictures should be done as soon as they are detected and while the patient is still on chemotherapy. Tuberculous strictures of the lower end of the ureter which cannot be dilated can be resected safely during chemotherapy.

f) Epididymal tuberculous abscesses respond well to chemotherapy. If the enlargement of the abscess progresses, or sinus formation occurs, or soreness persists, the nodule in the epididymis should be excised. The patient's fertility status should be considered, however, before irrevocable bilateral epididymectomy is performed. One year or more of chemotherapy should always accompany epididymectomy on the assumption that an active prostatic lesion is also present.

g) Prostatic and seminal vesicle tuberculosis also respond to chemotherapy, with "conversion" of urine cultures and some shrinkage in the size of the lesions. Total prostato-vesiculectomy is rarely indicated for prostatic tuberculosis.

h) Tuberculosis of the urinary bladder responds readily to chemotherapy if the lesions are superficial. If the bladder is scarred and severely contracted, however, diversion of the urine may become necessary in order to protect the kidneys from back pressure. Cutaneous ureterostomy is the operation of choice here. The ureters should never be transplanted into the colon when there is a question of active tuberculosis of the kidneys. The tuberculous kidney does not tolerate superimposed *B. coli* pyelonephritis.

i) Annual or semi-annual examinations for pyuria should be performed on all patients with tuberculosis of any form. As few as 3 to 5 leukocytes per high-power field in a centrifuged urine specimen (of specific gravity of 1.015 or less) should be investigated by repeated urine cultures and guinea pig inoculations for *M. tuberculosis*. Females should be catheterized. Tuberculosis may be present either with or without simultaneous pyogenic urinary tract infection, and with either alkaline or acid urine.

JOHN K. LATTIMER
For the Committee on Therapy

WILLIAM BARCLAY
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The Present Status of Excisional Surgery in the Treatment of Pulmonary Tuberculosis

A Statement of the Committee on Therapy

Since its statement of 1953, the Committee on Therapy has continued to follow changing trends in the indications for excisional surgery of pulmonary tuberculosis. The place of resection in the treatment of destroyed lung, tuberculous bronchiectasis, and fibrostenosis of a major bronchus has changed very little. The resection of cavities during prolonged chemotherapy, with or without positive sputum bacteriology, is generally practiced.

At the present time, the excision of nonair-containing nodules, either filled in or unsloughed, in patients with negative sputum bacteriology during prolonged antimicrobial therapy in pulmonary tuberculosis can neither be recommended nor condemned.

There is now less enthusiasm than formerly for such surgery because of the known results of bacteriologic studies of surgical specimens and the apparent success of long-term drug therapy.

Because of the antimicrobial therapy, advances in surgical technique and anesthesia, blood replacement, pulmonary function studies, improved patient care, and case selection, the surgical mortality and morbidity rates have been reduced to very low figures. Yet it should be remembered that the morbidity and mortality rates for long-term drug therapy are also very low.

When the patient is treated with long-term drug therapy with the addition of surgical excision, the physician should make every effort to conduct thorough morphologic and bacteriologic studies of resected tissues and all patients should be followed carefully, in order to increase our knowledge of this controversial subject.

Raymond F. Corpe
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NOTICES

ANNUAL MEETING OF THE AMERICAN TRUDEAU SOCIETY—1956

The American Trudeau Society, medical section of the National Tuberculosis Association, invites the submission of papers for the 1956 annual meeting to be held in New York City, May 21–25, 1956.

Abstracts

Abstracts of papers to be offered for presentation should be submitted before January 1, 1956, with an absolute deadline of January 21, 1956, to Dr. George C. Owen, Chairman of the Medical Sessions Committee, % the Wisconsin Anti-Tuberculosis Association, 1700 West Wells Street, Milwaukee 3, Wisconsin. *Six* copies are required for distribution to committee members. Receipt of abstracts will be acknowledged promptly.

Abstracts should be limited to a 300-word summary including important data and conclusions. Charts and tables should be attached to aid in the selection of papers.

The names, academic degrees, titles, and complete addresses of the senior author and co-authors should be included in each abstract. The author who is to present the paper must be the first author listed in the abstract.

All abstracts and papers will be reviewed by the Medical Sessions Committee and, when necessary, by experts in special fields in order to select those containing the most interesting material. Final selection will be on a competitive basis.

Subjects for presentation may include all scientific aspects of tuberculosis and non-tuberculous respiratory and cardiopulmonary diseases as well as the results of investigative work bearing on these subjects.

Those wishing to present subjects of a confidential nature from the armed forces or other sources must clear the material through official channels *before* submitting the abstracts to the Chairman of the Medical Sessions Committee.

Papers

Subsequent to review of the abstracts by the Medical Sessions Committee, papers will be chosen on a competitive basis from among the abstracts for presentation at the annual meeting. Authors will be notified by March 1, 1956, if their papers are to be read.

A double-spaced typewritten manuscript must be prepared in *duplicate* to be presented to the moderator of the session at the time the speaker presents his paper at the annual meeting.

Papers presented at the annual meeting must be original contributions which have not previously been presented or published. All such papers should be considered as belonging exclusively to the National Tuberculosis Association and should not be submitted for publication elsewhere without the advance permission of the National Tuberculosis Association.

All papers will be submitted to THE AMERICAN REVIEW OF TUBERCULOSIS AND PULMONARY DISEASES, but publication in the *Review* will be subject to the decision of the Editor. Exception will be made in the instance of papers concerned with work in progress, and designated both on the abstract and the manuscript as a Preliminary Report. Such papers need not be submitted for publication.

The time allotted for presentation of papers at the annual meeting will be strictly limited by the committee in order to permit adequate discussion.

Participants in the program are reminded of the fact that travel expenses to the annual meeting are not paid by the National Tuberculosis Association.

COURSE IN LABORATORY DIAGNOSIS OF TUBERCULOSIS

In cooperation with the Division of Special Health Services, Public Health Service, the Bacteriology Laboratories of the Communicable Disease Center, Chamblee, Georgia, will offer a course in the laboratory diagnosis of tuberculosis, November 14–25, 1955.

This course is open to all grades of employed laboratory personnel who are approved by their state health officers. Practical laboratory training in all phases of tuberculosis bacteriology, including preparation of culture media, microscopy, cultural procedures, diagnostic use of animals, and testing of drug sensitivity will be included in the course.

Laboratory directors and senior laboratory staff members may also make application for this course.

No tuition or laboratory fees are charged. Reservations for this course should be made well in advance.

Application forms may be obtained from Laboratory Training Services, Communicable Disease Center, Public Health Service, P. O. Box 185, Chamblee, Georgia.

FOURTH INTERNATIONAL CONGRESS AND FIFTH ARGENTINE CONGRESS OF BRONCHOESOPHAGOLOGY

The organizing Committee of the Fourth International Congress and Fifth Argentine Congress of Bronchoesophagology announces that the next Congress will be held in Buenos Aires, Argentina, on October 28 and 29. The following subjects will be considered: laryngotracheo-bronchopathology in children, stenosis and neoplasma of the esophagus; and miscellaneous subjects.

PULMONARY DISEASES—ORAL EXAMINATION

The American Board of Internal Medicine, One West Main Street, Madison 3, Wisconsin, wishes to announce to eligible candidates who wish to apply for admission that the Board in Pulmonary Diseases has scheduled an oral examination in pulmonary diseases in Louisville, Kentucky, on September 22–24, 1955.

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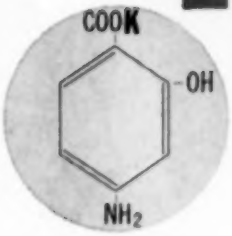

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1. *Molthan, L., Cohen, B. V., and Zarafonetis, C. J. D.: Clinical use of potassium para-aminosalicylate (KPAS). Am. Rev. of Tuberc. and Pulmon. Dis. 71:220 (Feb.) 1955.*

2. *Cohen, B. V., Molthan, L., and Zarafonetis, C. J. D.: Clinical Studies of Various Forms of PAS (with special reference to plasma concentrations). Read before the American Trudeau Society, Annual Meeting, Milwaukee, Wisconsin, May 23-27, 1955.*

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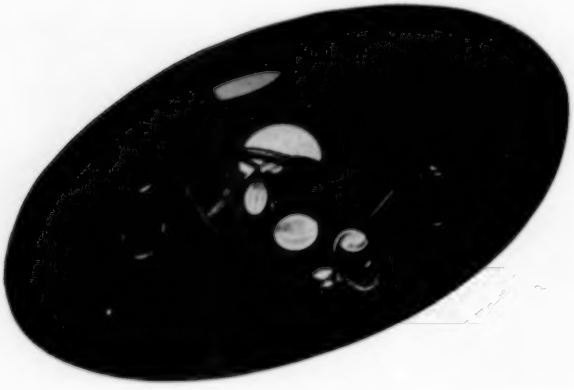

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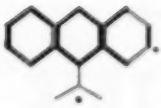

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1. Hyman, S., and Rosenblum, S. H.: Illinois M. J. 104:257, 1953.
2. Piper, C. E., and Nicklas, F. W.: Industr. Med. 23:510, 1954.

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